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**Diseases of wild birds of the orders Passeriformes and
Columbiformes - a review of conditions reported from
the United Kingdom and an analysis of results from wild
bird disease surveillance in Scotland 1994-2013**

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Diseases of wild birds of the orders Passeriformes and Columbiformes - a review of conditions reported from the United Kingdom and an analysis of results from wild bird disease surveillance in Scotland 1994-2013

Declaration

I declare that this thesis has been composed by myself, and that my input to the disease investigations was as described in the Materials and Methods. Some of the findings have been previously published in letters, short communications or papers in the scientific press, as referenced in the appropriate sections of the thesis. The body of work presented in this thesis has not been submitted for any other degree or professional qualification.



Thomas William Pennycott

29/01/16

ABSTRACT

There is growing concern about the impact of human activities on wildlife, both at the level of the individual animal and at a global population level, and the need for surveillance of wildlife for evidence of infectious and non-infectious diseases has never been greater. There is also much interest in attempting to help wildlife by treating and rehabilitating sick and injured wild animals and by providing supplementary feeding to garden birds. This thesis reviews the literature describing the diseases found in the United Kingdom (UK) in different birds of the orders Passeriformes and Columbiformes, the orders of birds with which members of the general public and wildlife rehabilitators are most likely to have contact. The thesis then collates and analyses the postmortem findings from wild bird surveillance carried out on 2048 birds of these orders at one diagnostic laboratory in Scotland over a twenty-year period (1994-2013). The overall aim was to make maximum use of surveillance data already gathered but not previously readily available, to inform those involved with wildlife disease surveillance, wildlife rehabilitation, and members of the public providing supplementary feeding to garden birds.

During the 20 years of wild bird disease surveillance, 42 endemic conditions or pathogens were identified, raising awareness and increasing our understanding of these conditions. One re-emerging disease, salmonellosis, came to prominence and then declined during the surveillance period, and was confirmed in approximately 350 garden birds. Two new conditions were described in finches; *Escherichia albertii* bacteraemia in approximately 150 finches and *Trichomonas gallinae* infection in approximately 370 finches. The large numbers of birds with salmonellosis, *E. albertii* bacteraemia or trichomonosis permitted further analysis by species of bird, geographic region, and distribution by age and sex, permitting conclusions to be drawn regarding the epidemiology of these diseases. Two new conditions were diagnosed in choughs (*Pyrrhocorax pyrrhocorax*), a species of conservation concern in the UK; a developmental abnormality of the eye and sometimes brain of young choughs, most

likely inherited, and significant helminthosis caused by spirurid gizzard worms and intestinal thorny-headed worms. These findings will influence future attempts to conserve this species in Scotland. Another new condition encountered was enteritis and/or hepatitis associated with schistosome-like eggs, diagnosed in blackbirds (*Turdus merula*) and a dunnoek (*Prunella modularis*). More specific identification of the causal organism and evaluation of potential zoonotic implications are required. Two conditions were investigated for which no satisfactory aetiological agent could be identified; a non-suppurative encephalitis affecting multiple fledgling starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*), and a necrotic oesophagitis of unknown cause detected in five chough nestlings. Three organisms identified in wild birds elsewhere in the world and found for the first time in the UK as part of this surveillance study were the avian gastric yeast *Macrorhabdus ornithogaster* (“megabacteria”) in greenfinches (*Chloris chloris*) and a waxwing (*Bombycilla garrulus*), *Mycoplasma sturni* in blackbirds, starlings and corvids, and *Ornithonyssus* sp. mites in corvids. Screening for two zoonotic pathogens exotic to UK wildlife, highly pathogenic avian influenza virus (HPAIV) and West Nile virus (WNV) was carried out on over 600 samples and over 500 samples respectively, but no positive results were obtained. Investigation of novel and re-emerging conditions and screening for exotic pathogens relied heavily on work carried out by other laboratories, underlining the importance of collaboration between multiple laboratories when carrying out disease surveillance.

To aid those working in wild bird disease surveillance, diagnosis and treatment, a collection of approximately 700 images of lesions, parasites and their eggs or oocysts is included as an appendix to this thesis, as has a guide to the presumptive identification of some of the internal parasites encountered.

This study has demonstrated the ever-changing nature of diseases of wild birds of the orders Passeriformes and Columbiformes, and the same is likely to be true of wild birds in other orders. Continued wild bird disease surveillance is essential, to help safeguard the health of wildlife, livestock, humans, and indeed the environment itself.

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List of abbreviations

AGY.....	Avian gastric yeast
AHVLA.....	Animal Health and Veterinary Laboratories Agency
AMR.....	Antimicrobial resistance
APHA.....	Animal and Plant Health Agency
APMV-1.....	Avian paramyxovirus-1
BAP.....	Biodiversity Action Plan
BBC.....	British Broadcasting Corporation
BGA.....	Brilliant Green Agar
BSAC.....	British Society for Antimicrobial Chemotherapy
BTO.....	British Trust for Ornithology
BWRC.....	British Wildlife Rehabilitation Council
CD.....	Cercarial dermatitis
CI.....	Confidence interval
CLDT.....	Cytolethal distending toxin
cm.....	centimetre
CNS.....	Central nervous system
CRS.....	Corvid respiratory syndrome
CTX-M.....	Cefotaximase
CVL.....	Central Veterinary Laboratory
DARDNI.....	Department of Agriculture and Rural Development for Northern Ireland
DCLS.....	Deoxycholate citrate lactose sucrose
Defra.....	Department for Environment, Food and Rural Affairs
DNA.....	Deoxyribonucleic acid
DSC.....	Disease Surveillance Centre
DT.....	Definitive phage type
e.g.	<i>exempli gratia</i> , for example
EHEC.....	Enterohaemorrhagic <i>Escherichia coli</i>
EID.....	Emerging infectious disease

ELISA.....	Enzyme-linked immunosorbent assay
EPEC.....	Enteropathogenic <i>Escherichia coli</i>
ESBL.....	Extended-spectrum beta-lactamase
Etc.	<i>et cetera</i> , and so forth
F.....	female
GB.....	Great Britain
GBHi.....	Garden Bird Health initiative
GWH.....	Garden Wildlife Health
H&E.....	Haematoxylin and eosin
HPAIV.....	Highly pathogenic avian influenza virus
I.....	Infectious
IBH.....	Inclusion body hepatitis
ICAHS.....	International Conference on Animal Health Surveillance
ICBP.....	International Council for Bird Preservation
IHC.....	Immunohistochemistry
IoZ.....	Institute of Zoology
JNCC.....	Joint Nature Conservation Committee
KOH.....	Potassium hydroxide
LA.....	Local Authority
LIMS.....	Laboratory Information Management System
LPAIV.....	Low pathogenic avian influenza virus
µg.....	microgram
µm.....	micrometre, micron
M.....	male
MAFF.....	Ministry of Agriculture, Fisheries and Food
MIC.....	minimum inhibitory concentration
MLST.....	Multi-locus sequence typing
MLVA.....	Multi-locus variable number of tandem repeats analysis
mm.....	millimetre
MMI.....	Mass mortality incident

MRI.....	Moredun Research Institute
MRSA.....	Meticillin-resistant <i>Staphylococcus aureus</i>
NERC.....	Natural Environment Research Council
NLF.....	Non-lactose fermenting
NOS.....	Not otherwise specified
NSE.....	Non-suppurative encephalitis
NSF.....	Non-sorbitol fermenting
OIE.....	World Organisation for Animal Health
p.	page (number)
PAFS.....	Predictable anthropogenic food subsidies
PAS.....	Periodic acid - Schiff
PCR.....	Polymerase chain reaction
PFGE.....	Pulsed field gel electrophoresis
PHLS.....	Public Health Laboratory Service
pp.	pages (numbers)
ppm.....	parts per million
PPMV-1.....	Pigeon paramyxovirus-1
PT.....	Phage type
PTFE.....	Polytetrafluoroethylene
R.....	Recovered
R ₀	Basic reproduction ratio, or Basic reproductive number
RAPD.....	Random amplified polymorphic DNA
RDNC.....	Reacts, does not conform
RNA.....	Ribonucleic acid
RSPB.....	Royal Society for the Protection of Birds
RSPCA.....	Royal Society for the Prevention of Cruelty to Animals
RT-PCR.....	Reverse transcriptase polymerase chain reaction
S.....	Susceptible
SAC.....	Scottish Agricultural College
SACCVS.....	SAC Consulting Veterinary Services

SASA.....	Science and Advice for Scottish Agriculture
Scottish SPCA.....	Scottish Society for the Prevention of Cruelty to Animals
S:I:R.....	Susceptible:Infectious:Recovered ratio
s.l.	<i>sensu lato</i> , in the broad sense
SNH.....	Scottish Natural Heritage
SOAEFD.....	Scottish Office Agriculture, Environment and Fisheries Department
SRUC.....	Scotland's Rural College
SSRL.....	Scottish Salmonella Reference Laboratory
ST.....	Sequence type
UK.....	United Kingdom
USA.....	United States of America
UV.....	Ultra violet
VAS.....	Veterinary and Advisory Services
VRE.....	Vancomycin-resistant enterococci
VTEC.....	Verocytotoxin-producing <i>Escherichia coli</i>
WIIS.....	Wildlife Incident Investigation Scheme
WNV.....	West Nile virus
WWF	World Wildlife Fund
ZN.....	Ziehl-Neelsen
ZSL.....	Zoological Society of London

Chapter 1

Wildlife – declines, supplementary feeding, rehabilitation and disease surveillance

1.1 Background

In 2014 the World Wildlife Fund (WWF) and the Zoological Society of London (ZSL) released The Living Planet Report (WWF and ZSL, 2014). This report revealed that the numbers of wild animals (mammals excluding humans, birds, reptiles, amphibians and fish) globally fell by over 50% between 1970 and 2010. Not only have population numbers halved, the number of different species of life forms is also declining at an alarming rate, possibly in the order of 1000 times the expected “natural” rate of species extinction (WWF 2015). Several reasons were given for the reduction in the number of species and the actual population sizes, many arising from the phenomenal success of one species, *Homo sapiens* (WWF and ZSL, 2014). The global human population has increased from under one billion (one thousand million) in 1800 to over seven billion in 2015, and is increasing by 200,000 each day (Geohive Regional Populations, 2015; World Population Live, 2015). This increase in the human population has been the most important factor in the reduction of wildlife populations, and the Living Planet Report (WWF and ZSL, 2014) attributed over 80% of this decline to human activities such as hunting, fishing, accidental bycatch and deliberate habitat degradation or change. Climate change, invasive alien species, pollution and disease were thought to have caused the remainder of the population losses.

Measures to address these global changes are out-with the control of most members of the general public in the United Kingdom (UK). However, three activities in which they can and do become involved with wildlife are the supplementary feeding of garden birds, the rescue and rehabilitation of sick and injured wildlife, and assisting with disease surveillance.

Wild birds are probably the wildlife that the general public have greatest interface with, especially garden birds, with population data for over 260 species in the UK provided by Musgrove et al. (2013). These authors estimated the breeding population of birds in the UK to be around 168 million, and by the autumn the overall number of wild birds rises to over 400 million birds as a result of surviving current-year birds, passage migrants and winter visitors. The provision of supplementary feed to garden birds, either throughout the year or only in the winter months, is one area in which there is considerable interaction between members of the general public and wildlife, and Section 1.2 below discusses this topic more fully. Data pertinent to garden bird mortality will be outlined in Chapters 3 to 11, and discussed in greatest detail in Chapter 7.

Another activity with which some members of the UK general public become involved is wildlife rescue and rehabilitation. Section 1.3 discusses some of the advantages and disadvantages of wildlife rehabilitation, and information that can inform wildlife rehabilitators is highlighted in Chapters 3 to 11.

Understanding the causes of mortality of wildlife is very important, whether in garden birds found dead at feeding stations, deaths in rehabilitation centres or after release, or birds found dead in the wider environment. One essential tool is surveillance, and members of the general public can assist in this by reporting sick or dead wildlife and in some circumstances by submitting carcasses for further investigation. Surveillance, literally “watching over”, can help to provide the data required to determine if individual deaths are due to human activities, pollution, infectious disease etc., and what the significance may be at a population level. Wild birds lend themselves to disease surveillance because they occupy a wide range of habitats, many species are easy to see and identify, and the general public are already interested in wild birds (Furness et al., 1993); in Section 1.4.3 of this chapter the benefits of carrying out disease surveillance in wild birds will be considered. In addition to concerns about the welfare of individual wildlife or implications at wildlife population levels, it must also be recognised that wildlife can sometimes have negative impacts on the health of humans, livestock and

pets. This aspect will be considered more fully in Section 1.4.3 below. Chapter 2 outlines the materials and methods used to carry out wild bird disease surveillance at Ayr Disease Surveillance Centre (DSC) between 1994 and 2013, and Chapters 3 to 11 detail the diseases found in wild birds of the orders Passeriformes and Columbiformes, the two orders of birds with which the UK general public are most likely to have contact. The major outcomes of wild bird disease surveillance at Ayr DSC between 1994 and 2013, and the implications for garden bird feeding and wildlife rehabilitation, are discussed in Chapter 12. For the purposes of wild bird disease surveillance and, where appropriate, for this thesis, birds were considered to be “wild” if they had not been bred in captivity and the species appeared on the checklist of British wild birds (Harrop et al., 2013). Feral birds such as feral pigeons (*Columba livia*) and feral eagle owls (*Bubo bubo*) were included, as were birds temporarily captive for the purposes of treatment and rehabilitation. Pheasants (*Phasianus colchicus*), red-legged partridges (*Alectoris rufa*) and red grouse (*Lagopus lagopus scoticus*) were excluded because these birds are managed for sporting purposes. Birds were described as “finches” if they belonged to the Family Carduelidae or Family Fringillidae, and “corvids” referred to birds of the Family Corvidae. The term “garden bird” was used to describe a species of wild bird that is included on the checklist of birds most often observed in gardens in the UK (Glue 1982).

1.2 Supplementary feeding of garden birds

1.2.1 Introductory comments

Across the UK, it has been estimated that approximately 12-13 million households feed garden birds, about half of all households (Davies et al., 2009). Around 60,000 tonnes of food are offered to garden birds in the UK each year (Fuller et al., 2008). Considering both the UK and the USA, Robb et al. (2008) and Plummer et al. (2013) suggested that these countries together provide over 500,000 tonnes of food for garden birds each year.

Feeding garden birds has many potential benefits, both for the birds themselves and for the humans providing the food. Possible benefits for the birds include greater

survivability and improved productivity, and human well-being is thought to be enhanced by greater connection with the natural world (Fuller 2008; Davies et al., 2009). However, there may also be a cost to the birds, in terms of greater exposure to pathogens and predators, changes in breeding and feeding strategies, and increased inter-species contacts. Some of the advantages and disadvantages of providing supplementary feed will be described below, and discussed more fully in Chapter 7.

1.2.2 Advice from the popular press

One of the first books to discuss ways of attracting and looking after birds in gardens was “*The Garden Bird Book*”, published in 1982 and edited by David Glue in collaboration with The British Trust for Ornithology (BTO). Glue (1982) noted that feeding garden birds in the UK increased after the end of World War 2, with the British Broadcasting Corporation (BBC) asking their listeners to provide food and water for wild birds during severe weather. Even in the early 1980s, however, there was debate about the ethics of feeding garden birds. Some of the issues discussed by Glue (1982) included the dangers of infectious disease, the consequences of maintaining abnormally large populations of common species which might compete with less-common species for food and nest sites, and the possibility that birds might become dependent on the food provided and be reluctant to disperse and forage elsewhere. There was also debate about whether birds should be fed in the winter only, or throughout the year: on the one hand it was acknowledged that the birds’ nutritional requirements greatly increased in the breeding and rearing seasons, but conversely there was concern that the artificial food provided might be unsuitable for this purpose. Other issues discussed by Glue (1982) included the potentially undesirable effects of artificially permitting weaker members of the wild bird population to survive, possibly adversely impacting on the population as a whole, and the likelihood of greater exposure to predators such as domestic cats, sparrowhawks (*Accipiter nisus*), corvids, and even great spotted woodpeckers (*Dendrocopus major*) that could feed on eggs and nestlings of other species in artificial nestboxes. On balance it was concluded that the advantages of

supplementary feeding outweighed the disadvantages, although the advantages (for the birds) were not fully explained.

When “*The Garden Bird Book*” was published in 1982, the commonest form of supplementary feeding was the provision of peanuts, either within the shell or more commonly as kernels in plastic nets or wire containers. Tits, greenfinches (*Chloris chloris*), house sparrows (*Passer domesticus*) and siskins (*Carduelis spinus*) had mastered the art of feeding at such containers but chaffinches (*Fringilla coelebs*) were said to have had relatively little success. Fat-balls, fruit, meat, grain-based foods and mealworms were also fed, and wild bird mixtures containing seeds such as sunflower, nyger, canary seed and hemp were being developed. Glue (1982) commented that house sparrows favoured bread and peanuts, and that greenfinches only visited gardens if wheat or peanuts were offered. Chaffinches, however, fed mainly on the ground and were said to rarely use bird feeders on a regular basis.

A little over 20 years later, The Wildlife Trusts produced “*The Garden Bird Handbook – How to Attract, Identify and Watch the Birds in Your Garden*” (Moss 2003). As in Glue (1982), a combined approach of habitat management and supplementary feeding was advocated, and the book highlighted an increase in garden bird feeding in the 1990s arising from advice by The Royal Society for the Protection of Birds (RSPB), BTO and The Wildlife Trusts, combined with the availability of a greater selection of wild bird foods from specialised suppliers. A move from peanuts to sunflower hearts and nyger seeds was noted, making supplementary food more attractive to species such as goldfinches (*Carduelis carduelis*) and chaffinches. Feeding in the autumn and winter was encouraged to increase winter survival, and spring/early summer feeding was advised to help adults during the breeding/rearing season. A reduction in feeding from mid-summer was suggested, due to the availability of other natural foods (Moss 2003). Predators and disease were identified as significant threats to the birds, but good hygiene was advised and most problems of disease were thought to be self-limiting. It was, however, recognised that supplementary feeding changed natural feeding behaviour, and

resulted in artificial competition between species that were normally ecologically separated.

Another book "*Garden Birds and Wildlife*", written by Mike Toms of the BTO and Paul Sterry, was published in 2008. Advice was again given to provide appropriate food throughout the year, not just in winter, to help the adults find sufficient food for themselves and their chicks. The dangers of feeding loose peanuts, salted food or desiccated coconut were highlighted, especially if then fed by the parents to the young birds. Supplementary feeding was said to increase winter survival and productivity, although it was acknowledged that the scientific evidence was based on studies in natural habitats rather than gardens. Toms and Sterry (2008) noted that black sunflower seeds and sunflower hearts had been fed to garden birds from the early 1990s, and nyger seeds more recently, attracting a wider range of bird species to garden feeders than previously. By 2008, the hazard of deaths in garden birds from infectious diseases such as salmonellosis and, especially, trichomonosis had become apparent, and Toms and Sterry (2008) advised a number of precautions that should be taken to reduce such risks. Daily brushing and regular cleaning and disinfection of bird tables and hanging feeders was advised. Moving hanging feeders regularly and cleaning underneath them, and if feeding from the ground, moving the feeding areas daily, was advocated to reduce a build-up of pathogens. Providing food at several locations in the garden was proposed to minimise the numbers of birds in one small area, and regular cleaning and rinsing of bird baths was also advised. The dangers of disease transmission were accepted, but the benefits in terms of improved survivability and productivity in the absence of disease were thought to outweigh the risks.

In 2009 the RSPB website advised feeding birds throughout the year, providing high protein food during the summer months. However, they cautioned that good hygiene was crucial, otherwise more harm than good might result (RSPB 2009).

1.2.3 Scientific studies evaluating supplementary feeding

Suggested benefits of supplementary feeding have included earlier laying of eggs, larger clutches, reduced incubation times and greater hatching and fledging success (Robb et al., 2008). These authors, providing peanut feeders to blue tits (*Cyanistes caeruleus*) in woodland sites, found that birds on sites fed over the winter had advanced laying dates and increased numbers of chicks fledging. Nevertheless, they warned that there could also be adverse effects, in particular the danger that artificially-induced earlier laying might result in young birds in the nest before the peak of natural food abundance occurred. They described this possibility as an “*ecological trap*”, adversely affecting the ability of birds to judge the best time and place to breed. Harrison et al. (2010), after providing supplementary food in the form of peanut cake to blue tits and great tits (*Parus major*) in woodland, found that supplementation from pre-laying to hatching advanced laying and shortened incubation time, but in their study also reduced brood size in both species. In the case of great tits, smaller clutches were laid, and in blue tits clutches were smaller and hatching success was reduced. Another study involving blue tits, this time fed on vegetable fat with or without vitamin E during the winter, also found that supplemented birds had significantly lower productivity (smaller chicks, lower survival rates) than non-supplemented birds (Plummer et al., 2013), possibly by helping poorer quality birds to survive the winter and then breed. More work is required to clarify the long-term effects of supplementary feeding on breeding productivity.

Feeding garden birds in winter in the UK was shown to significantly increase the likelihood that certain species of birds would visit the garden (Chamberlain et al., 2005). Collared doves (*Streptopelia decaocto*), magpies (*Pica pica*), greenfinches, woodpigeons (*Columba palumbus*), jackdaws (*Corvus monedula*), chaffinches and feral pigeons showed the greatest increases in probability of occurrence. Fuller et al. (2008) found that avian abundance was strongly linked to the density of garden bird feeders in an area, although these authors found no effect on species richness. However, Oro et al. (2013) argued that the intentional provision of food in a manner that is predictable in terms of time and location (predictable anthropogenic food subsidies ([PAFS]) could

increase the population of resilient species to the detriment of other species, effectively reducing the overall diversity of the community. Similarly, the potential for supplementary feeding to have adverse effects on the composition of bird species visiting gardens in New Zealand was highlighted by Galbraith et al. (2015). Providing bread and seeds encouraged species such as house sparrows and spotted doves (*Streptopelia chinensis*) to visit gardens, but possibly at the expense of other species such as the grey warbler (*Geryone igata*), an insectivorous bird feeding in trees. This was of particular concern because those species that thrived were introduced species, whereas the grey warbler is native to New Zealand. McKinney (2002), reviewing the impacts of urbanisation on wildlife in the USA, also noted that the number of non-native species (e.g. house sparrows and starlings [*Sturnus vulgaris*]) increased and the number of native species declined with increasing urbanisation.

The effects of attracting wild birds from a rural environment to an urban location have attracted scientific interest in recent years, and Chamberlain et al. (2009) conducted a review and meta-analysis of the implications of increasing urbanisation across an urban-rural gradient. Variation between species was noted, but in general birds in urban landscapes started to lay earlier, had smaller clutches, their nestlings weighed less, and productivity per nesting attempt was poorer. Conversely, there was improved winter survival of adults, leading to greater breeding densities, and for species producing multiple broods the increase in nesting attempts compensated for the lower productivity of each nest. Possible increased hazards arising from greater urbanisation included reduced food availability for the chicks, more predation, pollution, increased noise and light levels, infectious diseases, and collisions with man-made structures (Chamberlain et al., 2009). These authors concluded that there was no evidence that the urban environment constituted an “ecological trap”, but that major differences were observed between species and that increased monitoring across a wide range of species was required before the effects of urbanisation could be fully understood.

A more unusual and controversial form of wild bird feeding, namely putting out meat in gardens to attract red kites (*Milvus milvus*), was discussed by Orros and Fellowes (2014). The main motivation for feeding these large re-introduced raptors was said to be a desire to see them at close range, followed by the hope that feeding red kites would help in their conservation. These authors noted some of the concerns that had been raised about feeding red kites, including uncertainty about the nutritional value of cooked meat, and the possibility of metabolic bone disease in young birds if the skin and bones had been removed from the meat prior to feeding. Other potential issues were that supplementary feeding of re-introduced red kites might delay their subsequent dispersal and colonisation, and that other species of birds might be discouraged from using these feeding sites. A summary of the advice given by various organisations to reduce potential adverse effects was provided by Orros and Fellowes (2014).

1.2.4 Infectious diseases at garden bird feeding stations

Probably the biggest negative impact of feeding garden birds is infectious disease, a hazard recognised for many years. Macdonald and Cornelius (1969) suggested that salmonellosis in greenfinches and house sparrows resulted from a build-up of pathogens at bird tables, and Pennycott et al. (1998) drew similar conclusions for salmonellosis and *Escherichia albertii* bacteraemia in finches. Conjunctivitis in house finches (*Carpodacus mexicanus*) in the USA caused by *Mycoplasma gallisepticum* was associated with the use of bird feeders (Hartup et al., 1998), and two other conditions of wild birds spread at bird feeding stations are trichomonosis (Lawson et al., 2012b) and pox (Lawson et al., 2012a). These conditions and the possible role of supplementary feeding are discussed more fully in Chapters 3 to 7.

1.2.5 Garden bird feeding in Scotland 2005-2008

Information on the pattern of wild bird feeding in Scotland was obtained from questionnaires completed by members of the Garden Bird Health initiative (GBHi) that submitted carcasses to Ayr DSC between May 2005 and March 2008 (GBHi unpublished data). Of 94 locations, 86 fed throughout the year, five in winter only, and three were

variable. Food was offered daily on 87 sites, every second day on one site, and weekly on six sites. Fifty sites provided 500-2000g of food daily, and on six sites over 2000g of food was provided daily. The commonest foods presented were peanuts (regularly provided on 73 locations), mixed wild bird seeds (regularly offered on 66 sites), sunflower seeds (50 sites) and sunflower hearts (39 sites). Although these sites are unlikely to be representative of all households in Scotland, the results illustrate the heavy commitment to supplementary feeding undertaken on some sites in Scotland.

1.2.6 Alternative ways of providing food to garden birds

In light of some of the disadvantages discussed above, there is interest in developing “wildlife-friendly” gardens that provide access to natural sources of food. Glue (1982) considered that gardens should be designed and managed to provide food and water, shelter, nest sites and roosts for garden birds. Key elements identified were: trees, hedges and shrubs that produce fruit or seeds, or that attract insects that birds can feed on; shrubs providing ground cover; lawns; compost heaps; and ponds. Trees and shrubs that produce fruit and smaller berries provide food for blackbirds (*Turdus merula*), song thrushes (*T. philomelos*), starlings, robins (*Erithacus rubecula*) and more unusual birds such as waxwings (*Bombycilla garrulus*). Seed-producing trees and plants offer a source of food for birds such as finches, and flowering plants that attract invertebrates indirectly benefit insectivorous birds such as blue tits and great tits. The book “*Birds in Your Garden*”, produced jointly by The Royal Horticultural Society and The Wildlife Trusts in 2007 (RHS/WT 2007), noted that wildlife-friendly gardens provided corridors that wildlife could use to travel between rural, suburban and urban habitats, and provided lists of suitable plants that produced seeds, fruit or nectar throughout the year. This book also noted that the manner in which gardens were managed could impact on wild birds: restricting the use of insecticides, slug pellets and weedkillers would be beneficial, as would delaying the cutting back of annual plants and herbaceous shrubs and the removal of leaf litter, dead wood and other debris. Making maximum use of natural food resources, in gardens and on farmland, was advocated by workers concerned about the possible spread of trichomonosis among turtle doves (*Streptopelia turtur*) feeding at

grain piles artificially provided on farmland in England (Anon 2015; Stockdale et al., 2015).

1.3 Wildlife rehabilitation

Wildlife rehabilitation has been defined by the International Wildlife Rehabilitation Council as *the managed process whereby a displaced, sick, injured or orphaned wild animal regains the health and skills it requires to function normally and live self-sufficiently* (Molony et al., 2007). In 2011 in the UK, at least 71,000 wildlife casualties were admitted to wildlife establishments (Grogan and Kelly, 2013). Kirkwood and Sainsbury (1996) considered that in theory wildlife rehabilitation was carried out either for perceived welfare reasons or for conservation purposes, but in practice in the UK the latter was seldom of significant relevance.

Several authors (e.g. Kirkwood and Sainsbury, 1996; Kirkwood and Best, 1998; Joys et al., 2003; Kirkwood 2003; Molony et al., 2007; Grogan and Kelly, 2013; Meredith 2014) have made the point that any attempts at rehabilitation must not jeopardise the welfare of the individual animal or bird. Potentially adverse impacts on welfare could arise from the stress of capture, examination, treatment, confinement and release; extended pain and distress by keeping the patient alive; and effects on other animals in the rehabilitation centre or after release, including the introduction of disease (Kirkwood and Sainsbury, 1996). Another concern expressed by these authors was the possibility that the rehabilitation process might interfere with the natural evolutionary selection by which only the fittest animals survive and produce offspring.

The process of wildlife rescue, rehabilitation and release could also adversely affect the health of farmed livestock. Alexander et al. (2010) summarised the incursions of highly pathogenic avian influenza subtype Asian H5N1 into Great Britain (GB) between 2005 and 2008, and noted that two incidents involved wild swans. In one incursion, a dead whooper swan (*Cygnus cygnus*) was washed up on the east coast of Scotland, and in the other incident ten mute swans (*C. olor*) and a Canada goose (*Branta canadensis*) were

found dead over several weeks at a location on the south coast of England. If these birds had been found alive but unwell, and taken to a wildlife rehabilitation centre, further spread of HPAIV could have occurred within the centre, including to birds of different species due to be released back into the wild. Virus could then spread to poultry flocks, causing substantial problems to the national poultry industry in general and to affected poultry flocks in particular. The same scenario could occur with Newcastle disease caused by pigeon paramyxovirus-1, a virus previously found in feral pigeons and that spread to poultry flocks (Alexander et al., 1984; Lister et al., 1986).

Nevertheless, where the injuries or disease arose from the actions of humans, there might be grounds for attempting treatment and rehabilitation, provided the welfare of the animal or bird was not compromised (Kirkwood and Sainsbury, 1996; Kirkwood and Best 1998). Some of the potential benefits of attempting rehabilitation in wildlife were listed by Kirkwood (2003) and included the restoration of the health of the patient; training for those involved with rehabilitation; promotion of wildlife protection by the general public; wildlife disease surveillance; and well-being for humans interacting with wildlife.

There have been relatively few studies to show how successful or otherwise the rehabilitation process is. A survey by the British Wildlife Rehabilitation Council (BWRC) suggested that 39-53% of common species of birds submitted to rehabilitation centres between 1993 and 1995 were subsequently released (Kirkwood and Best, 1998), and Kirkwood (2003) quoted a figure of 47% of birds in 2000. In a review of studies carried out at four RSPCA wildlife hospitals, Grogan and Kelly (2013) quoted release figures (excluding birds euthanased or dying within 24 hours of admission) of only 24% of sparrowhawks, 31% of juvenile woodpigeons and 14% of adult woodpigeons. Joys et al. (2003) cautioned that the release of birds did not equate to successful rehabilitation, and stated that *for rehabilitation to be classed as successful from an individual's standpoint, it must be re-established back into the wild population and have a similar chance to those of wild birds of entering the breeding pool*. Using ring recoveries of

rehabilitated and released wild birds, Joys et al. (2003) concluded that post-release survival of mallard (*Anas platyrhynchos*), mute swans, buzzards (*Buteo buteo*) and sparrowhawks was relatively good, but for gannets (*Morus bassanus*), herring gulls (*Larus argentatus*), little owls (*Athene noctua*) and barn owls (*Tyto alba*), post-release survival was relatively poor. The report noted that for some species such as common scoters (*Melanitta nigra*) and guillemots (*Uria aalge*), post-release survival was so poor that the great majority were unlikely to enter the wild population. Sample size, however, was low, and these authors urged rehabilitators to ring as many birds as possible prior to release.

Whether to attempt treatment and rehabilitation of an individual, or euthanase on welfare grounds, is clearly not always an easy decision to make, and Meredith (2014) presented a number of factors to be considered in this situation. Key aspects of the patient included species, age, sex and natural biology. The nature of the injury or disease should be considered, including the cause, severity, the likely duration of treatment and frequency of handling, and the risks to personnel, other wildlife and livestock during rehabilitation and after release. Practical considerations such as the availability of treatment and rehabilitation facilities, suitable release sites, time of year and legal aspects should all be taken into account (Meredith 2014).

There is currently (January 2016) no requirement for wildlife rehabilitation centres to be licensed, although such an approach would be favoured by the Royal Society for the Prevention of Cruelty to Animals (RSPCA), who have expressed concern about the current lack of regulation (RSPCA 2015). Similarly, in response to a consultation document circulated by the Scottish Government, 76 of 91 individuals or organisations, including the Scottish SPCA and the Scottish Branch of the British Veterinary Association, agreed that “sanctuaries”, including wildlife rehabilitation centres, should be licensed (Scottish Government 2005). Reasons given included protection from neglect and suffering, and prevention of disease.

Nevertheless, the Animal Welfare Act (2006) in England and Wales (http://www.legislation.gov.uk/ukpga/2006/45/pdfs/ukpga_20060045_en.pdf, accessed 06/01/16), and The Animal Health and Welfare (Scotland) Act 2006 (http://www.legislation.gov.uk/asp/2006/11/pdfs/asp_20060011_en.pdf, accessed 06/01/16), have placed a burden of responsibility on those who care for wildlife in rehabilitation centres, including the need to protect wildlife undergoing rehabilitation from pain, suffering, injury and disease. In their document *Establishment Standards for Wildlife Rehabilitation*, produced to assist in compliance with the Animal Welfare Acts, the RSPCA highlighted the risks posed by cross-infection with parasites and other infectious diseases within premises, and advised that the cause of death of wildlife dying after admission to the centre be established and recorded (RSPCA 2010). Marking and post-release monitoring of rehabilitated animals was also recommended, to assess post-release survival.

Wild bird disease surveillance, including the examination of birds that die or are euthanased in rehabilitation centres and post-release, should, therefore, be an integral component of wildlife rehabilitation, and the results generated would help rehabilitators and their veterinary advisers make informed decisions about the best courses of action to be taken in the future.

1.4 Disease surveillance

1.4.1 Introduction

The importance of surveillance for animal health and disease is well recognised throughout the world, but the descriptive terms used (disease surveillance, disease monitoring, active surveillance, passive surveillance, scanning surveillance, targeted surveillance etc.) have often been poorly defined. A series of workshops of the International Conference on Animal Health Surveillance (ICAHS) resulted in broadly-agreed definitions for patterns of disease occurrence and methods of animal health surveillance (Hoinville et al., 2013). These authors also summarised the main purposes of animal health surveillance or monitoring, and the actions that might be taken by

policy-makers based on surveillance information received. Unless stated to the contrary, the definitions used below are as reported by Hoinville et al. (2013).

1.4.2 Definitions

Patterns of disease occurrence

Table 1.1 lists the definitions used when describing the patterns of disease occurrence, mostly based on the terms agreed in Hoinville et al. (2013).

Table 1.1: Patterns of disease occurrence. (From Hoinville et al. [2013]).

Terminology	Definition
Endemic	Constant presence of a known disease in the population of interest.
Sporadic	A known disease that occurs intermittently or in an irregular or haphazard fashion.
Exotic	A previously defined (known) disease that crosses political boundaries to occur in a country or region in which it is not currently recorded as present.
Re-emerging	A previously defined (known) disease that was either absent or present at a low level in the population in a defined geographical area, but that re-appears or significantly increases in prevalence.
New (emerging)	A previously undefined (unknown) disease or condition, which might result from the evolution or change in an existing pathogen or parasite. This term also applies to the emergence of any other previously undefined condition.
Epidemic	Not defined by Hoinville et al. (2013). The occurrence of more cases of disease than expected in a given population or among a specific group over a particular period of time (Centre for Disease Control definition).
Emerging infectious diseases (EIDs)	Not defined by Hoinville et al. (2013). Disease-causing agents that rapidly increase in geographical range, host range or prevalence (Tompkins et al., 2015).

Different forms of surveillance and monitoring

Table 1.2 lists the definitions used when describing different forms of disease surveillance or monitoring, based on the terms agreed in Hoinville et al. (2013). For the purposes of this thesis, the terms “surveillance” and “monitoring” will be considered interchangeable.

Table 1.2: Terminology used when describing different forms of surveillance or monitoring. (From Hoinville et al. [2013]).

Terminology	Definition
Animal health surveillance	The systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation and timely dissemination of animal health- and welfare-related data from defined populations. These data are essential for describing health hazard occurrence and to contribute to the planning, implementation and evaluation of risk mitigation measures.
Animal health monitoring	The definition of “animal health monitoring” is broadly similar to “animal health surveillance” but implies that there is no pre-defined action plan. The systematic (continuous or repeated) measurement, collection, collation, analysis and interpretation of animal health- and welfare-related data in defined populations, when these activities are not associated with a pre-defined risk mitigation plan. Extreme changes may, however, lead to actions being taken.
Active (proactive) surveillance	Investigator-initiated collection of animal health-related data through actions scheduled in advance using a defined protocol. Decisions about whether information is collected, and what information should be collected from which animals, is made by the investigator. One form of active surveillance is hazard-specific (targeted) surveillance.
Passive (reactive) surveillance	Observer-initiated provision of animal health-related data (e.g. voluntary notification of suspect disease) or the use of existing data for surveillance. Decisions about whether information is provided, and what information is provided from which animals, is made by the observer.
Enhanced passive surveillance	Observer-initiated provision of animal health-related data but with active investigator involvement, e.g. by encouraging reports of certain types of disease or by active follow-up of suspect disease reports, with fine-tuning by the investigator to standardise and make better use of the information obtained. One form of enhanced passive surveillance is early-warning or scanning surveillance.
Early-warning surveillance	Surveillance of health indicators and diseases in defined populations to increase the likelihood of timely detection of undefined (new) or unexpected (exotic or re-emerging) threats. Previously early warning surveillance was referred to as “scanning surveillance”, and is a form of passive surveillance.
Hazard-specific surveillance	Surveillance focused on specific pathogens or hazards, including known (endemic, re-emerging or exotic) diseases. Also referred to as “targeted surveillance”. A form of active surveillance, often developed when new information about a pathogen has emerged (OIE 2011). Can be used to demonstrate freedom from/presence of a pathogen or disease, or identify changes in its occurrence or distribution (OIE 2011).
Sentinel surveillance	Repeated collection of data or samples from selected sites that act as proxies for the entire population. Or efforts directed towards animals or premises that provide an increased probability of detecting a disease if present.

Collaboration between members of the general public and members of the scientific community has been termed “citizen science”, and can play a major role in wildlife disease surveillance (Lawson et al., 2015c). These authors summarised the advantages of citizen science involvement, including cost-effectiveness, access to data from a wide geographic area and for prolonged periods of time, and the opportunity to collect additional data such as the number of birds visiting a particular site. The general public may become involved by reporting sick or dead wildlife, by submitting carcasses for postmortem examination, and in some situations by collecting and submitting samples from wildlife. Those working at rehabilitation centres could play a similar and valuable role (Lawson et al., 2015c). Limitations noted included the risks of inaccuracy and bias in the data collected, but inaccuracy could be minimised if the role of the citizen scientist was to submit carcasses rather than make subjective observations, and the implications of bias in the selection of the carcasses submitted could be addressed when interpreting the results.

1.4.3 Why carry out disease monitoring/surveillance in wild birds?

Wild birds as reservoirs of endemic pathogens or diseases

Early investigations from the 1930s to 1960s into the causes of death of wild birds in the UK were often carried out to look for infectious diseases that could spread to poultry. Macdonald (1962b) commented that this had been the case at Lasswade Veterinary Laboratory in Scotland since the laboratory opened in 1939, and cited Newcastle disease in a gannet and in shags (*Phalacrocorax aristotelis*) as examples of the value of this approach. The occurrence of the gapeworm *Syngamus trachea* in wild birds was investigated by Campbell (1935) because of risks of its spread to poultry, and the role of wild birds such as starlings and rooks (*Corvus frugilegus*) in transmitting *S. trachea* and other helminths to domestic poultry was further pursued by Clapham (1940) while working at the Institute of Agricultural Parasitology, St. Albans. Concerns about the possible spread of disease from wild birds to poultry and to other livestock was also the reason given by Keymer (1958) for examining the carcasses of over 500 wild birds at the

Central Veterinary Laboratory (CVL), Weybridge, between 1954 and 1957. Although non-infectious causes such as poisoning and trauma predominated, pathogens that could spread to poultry included *Pasteurella multocida*, *Yersinia pseudotuberculosis*, *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S. Typhimurium*), *Mycobacterium avium*, and avian poxvirus. Another survey by Keymer and his colleagues at CVL focused on parasites of wild birds (including gamebirds) that might spread to domestic poultry (Keymer et al., 1962); 16 different species of helminths and six species of ectoparasites that had previously been recorded in or on poultry were identified in or on wild birds, including the gapeworm *S. trachea*, the red mite *Dermanyssus gallinae* and the hen flea *Ceratophyllus gallinae*.

McDiarmid (1956) of the Agricultural Research Council, Compton, described his investigations into the causes of death of wild birds in Berkshire between 1946 and 1956, looking for diseases that could spread to livestock and also to humans. Important examples given by McDiarmid included avian tuberculosis in wild birds that could spread to poultry and to cattle, and infection with *Y. pseudotuberculosis* and with what is now recognised as *Chlamydia (Chlamydophila) psittaci* with the potential for spread to humans. Also working in this field of work were Jennings and Soulsby at the Department of Animal Pathology, University of Cambridge, who examined approximately 1000 wild birds between 1954 and 1958. Their findings were summarised by Jennings (1961), who noted that the major causes of death were trauma (327 birds), parasitic diseases (117 birds), poisoning (96 birds), bacterial diseases (78 birds) and viral diseases (62 birds).

Surveillance for pathogens in UK wild birds continued from the 1960s to the 1990s, with the focus being on *Salmonella* spp. (e.g. Wilson and Macdonald, 1967; Goodchild and Tucker, 1968; Macdonald and Cornelius, 1969; Mitchell and Ridgwell, 1971; Johnston et al., 1979; Girdwood et al., 1985; Monaghan et al., 1985; Pennycott et al., 1998). Reviewing the extent to which wild animals and birds acted as reservoirs of infectious agents, Simpson (2002) included *D. gallinae*, *Mycobacterium avium*, *Campylobacter*

spp., *Salmonella* spp., *Borrelia burgdorferi* (the cause of Lyme disease), *C. psittaci*, Newcastle disease virus and avian influenza virus, as important pathogens that could spread from wild birds to livestock, pets or humans.

The importance of monitoring wildlife for potential pathogens was formally accepted in Scotland in 1994 (see 2.2 of this thesis) and in 1998 in England and Wales (Duff et al., 2010). Knowledge of the pathogens carried by wild birds will help to determine the epidemiology of disease outbreaks in livestock, pets and humans, and permit the implementation of appropriate biosecurity measures at local, national or international levels.

Wild birds as reservoirs of new or re-emerging diseases, exotic diseases, and “emerging infectious diseases” (EIDs)

New diseases, re-emerging diseases and exotic diseases (see Table 1.1 for definitions) may be detected in wild birds as a result of early-warning surveillance. Provided such diseases remain sporadic they may have limited impact on wild bird populations, humans, livestock or pets. However, if they become “emerging infectious diseases” (EIDs), defined by Tompkins et al. (2015) as *disease-causing agents that rapidly increase in geographical range, host range or prevalence*, then their significance can greatly increase. A statistic commonly quoted, from Jones et al. (2008), is that of 335 EIDs in recent decades, 60% were zoonotic, and of these, 72% originated in wildlife. Tompkins et al. (2015) expressed concern that in some cases there was insufficient robust evidence to meet the definition of a wildlife EID, and carried out a review of possible EIDs reported from 2000 onwards. Seventy potential EIDs of wildlife were identified (9 in amphibians and reptiles, 8 in birds, 18 in eutherian mammals, 7 in marsupials and monotremes, and 28 in fish), and in nearly half there was good supporting evidence that the definition of an EID had been met. Of the eight potential EID pathogens in birds, five were considered to have fulfilled the criteria – highly pathogenic avian influenza virus (HPAIV) H5N1, West Nile virus (WNV) lineage 1, WNV lineage 2, Usutu virus, and *Mycoplasma gallisepticum*. It is worth noting that the

first four pathogens are also capable of causing significant disease in humans. The remaining three potential avian EIDs (trichomonosis, avian pox and avian cholera) were discounted because the causal organisms were already widespread in birds. Tompkins et al. (2015) noted that for some putative wildlife EIDs that they reviewed, there was insufficient evidence that the disease or pathogen had previously been absent or was rapidly increasing in geographical range, host range or prevalence, and they recommended increased surveillance of wildlife to detect and enable better management of new EIDs.

The importance of the link between diseases of wildlife and diseases of humans has been recognised for many years, and in 2004 was encapsulated in the term “One World, One Health”. At a meeting of the Wildlife Conservation Society in New York in September 2004, the relationship between the health of humans, livestock, wild animals and the environment was emphasised, as was the need for an international and interdisciplinary approach to the prevention of epidemic disease and the protection of ecosystem integrity (Wildlife Conservation Society 2004). One of the so-called Manhattan Principles arising from this meeting was the need to provide adequate resources for wildlife health surveillance to provide early warning of disease threats. Wild bird disease surveillance as discussed in this thesis therefore supports the principles of One World, One Health, now abbreviated to “One Health”. However, concern has been expressed that too much emphasis has been put on the zoonotic implications of spill-over of disease from wildlife to humans, and not enough on wildlife conservation, biodiversity, socio-economic and environmental aspects (Buttke et al., 2015; Jenkins et al., 2015). Over-emphasis of the zoonotic risks of the human-wildlife interface could have a negative impact on the way in which the general public regard wildlife, and Buttke et al. (2015) advised caution in the manner in which details of wildlife-associated diseases were presented to the general public. Those involved with wild bird disease surveillance can assist in this by not exaggerating the hazard to humans should a potentially zoonotic pathogen be detected in a sporadic fashion.

Direct effect of pathogens on wild bird populations

In one of the early reports summarising the causes of mortality in wild birds, Jennings (1955) speculated that infectious diseases could directly or indirectly play a role in controlling the population size of wild birds. Of 224 birds examined, infectious diseases such as coccidiosis, avian tuberculosis, salmonellosis, trichomonosis, pasteurellosis, listeriosis, aspergillosis and avian pox accounted for the deaths of 70 birds. For many years this view was not widely shared, but in 1989 a Technical Publication from the International Council for Bird Preservation (ICBP 1989) concluded that there was increasing evidence that infectious and parasitic diseases could adversely affect the health of avian populations, and that health monitoring should be carried out on endangered species. Around the same time, the Natural Environment Research Council (NERC) noted that disease in wildlife might have ecological consequences, and carried out a review into the investigation of wildlife disease in the UK (Osborn et al., 1990). One of their recommendations was that a programme of research be carried out to improve the understanding of the impact of disease on wildlife, although no such programme materialised.

Kirkwood (1993) noted increased interest in the role of clinical and sub-clinical infectious disease on the lifetime reproductive success and mortality of wildlife, and in 2005 the possibility was raised that infectious diseases might be adversely affecting the health of garden birds being provided with supplementary feed at bird feeding stations (Cunningham et al., 2005). This resulted in additional disease surveillance being carried out in garden birds (the Garden Bird Health Initiative) and shortly afterwards deaths from trichomonosis were diagnosed in finches in gardens (Pennycott et al., 2005c). Continued wild bird surveillance conclusively demonstrated that within two years of its appearance, this infectious disease had reduced the breeding population of greenfinches in GB by 35% and chaffinches by 21% (Robinson et al., 2010). By 2009 the breeding population of greenfinches in GB had fallen by about 1.5 million birds (Lawson et al., 2012b). Finch trichomonosis is discussed in full in Chapter 6 of this thesis. Early recognition of the emergence of this novel form of trichomonosis was only possible

because active and passive surveillance systems were already in place when the condition appeared, and mitigation measures were swiftly proposed and disseminated by way of postmortem reports, press releases and newsletter articles. The possible impact of infectious diseases on wild bird populations, and therefore the need for enhanced surveillance, would be even greater if the species involved was included in the UK Biodiversity Action Plan (JNCC 2012) because its numbers or habitat were threatened.

Accidental or deliberate harm to wild birds

Another reason for carrying out disease monitoring/surveillance in wildlife is to look for evidence of harm done, deliberately or accidentally, by humans to wildlife. Concerns about deaths in wildlife arising from the use (approved use, misuse, or deliberate abuse) of pesticides lead to the establishment of the Wildlife Incident Investigation Scheme (WIIS). This scheme provides a means of post-registration surveillance of pesticide use and the results may provide feedback into the regulatory process. Fletcher (1994) described 85 investigations in waterfowl between 1982 and 1991, 28 of which were confirmed as agrochemical poisoning, and Johnson (1996) summarised the findings in over 450 agrochemical poisoning incidents in wild mammals and birds in Britain between 1990 and 1994. In England WIIS is run by Natural England (on behalf of the Health and Safety Executive), in Scotland by Science and Advice for Scottish Agriculture (SASA), in Wales by the Welsh Government, and in Northern Ireland by the Department of Agriculture and Rural Development for Northern Ireland (DARDNI).

Some major welfare problems arising in wild animals and birds as a result of human activities were reviewed by Sainsbury et al. (1995) and included lead poisoning in waterfowl caused by the ingestion of spent shotgun pellets; collisions with vehicles; predation by domestic cats; the physical and toxic effects of oil spills and other environmental pollutants; entanglement in fishing gear; and problems arising from wildlife rehabilitation and release. Other human activities that could harm wild birds are the erection of wind turbines (e.g. Everaert and Stienen, 2007; Saidur et al., 2011) and

the fitting of harness-mounted radio transmitters to wild birds for research purposes (Peniche et al., 2011).

Wild birds as biological monitors of the environment

The use of living organisms or their carcasses to monitor the environment was discussed in detail by Furness et al. (1993). Environmental changes could result from climatic changes; changes in economic activities (farming, forestry, fisheries etc.); habitat alteration/fragmentation; introduced alien species; and pollution (Furness et al., 1993). Wild birds were considered to be good monitors of the environment because they were easy to identify, occupied a wide diversity of habitats and trophic levels, some species were long-lived, and there was already considerable public interest in wild birds. Surveillance data could include species abundance, reproduction, survival, and tissue levels of pollutants, pesticides, rodenticides and radio-nucleotides (Furness et al., 1993). Wild birds have also been used in some countries such as the USA as early indicators of the presence of WNV lineage 1, on the basis that deaths in wild birds precede clinical disease in humans (Phipps et al., 2008). However, in Europe, where WNV lineage 2 predominates, this approach may be less successful because mortality in wild birds from WNV lineage 2 is not as high as from lineage 1.

Another scheme that uses wild birds to monitor the environment is the Predatory Bird Monitoring Scheme, a UK-wide programme operated by the Centre for Ecology and Hydrology of NERC (Walker et al., 2008). Concentrations of selected pesticides and pollutants in the liver and eggs of predatory birds are determined to provide information about exposure to such chemicals. Walker et al. (2008) also identified other chemical threats to species of high conservation status and discussed mitigation measures that have been taken in response to some incidents. Another example is the analysis of blood samples from waterfowl to detect recent exposure to lead from the environment, most commonly from the ingestion of spent lead gunshot (Newth et al., 2013). Two more examples are hazard-specific surveillance of fulmars (*Fulmarus glacialis*) for the presence of plastic in their stomachs, as indicators of changes in the abundance of plastic

litter in the marine environment (van Franeker et al., 2011), and rodenticide levels in the livers of raptors as indicators of secondary exposure to anticoagulant rodenticides (Hughes et al., 2013). Data obtained from wild bird surveillance schemes such as those described above can then be used by policy-makers to put in place measures to address the issues that have been identified.

Statutory surveillance of wild birds for avian influenza viruses

Following the spread of HPAIV H5N1 from south-east Asia into Europe in 2005, European legislation required that Member States carry out targeted surveillance for avian influenza viruses. This was deemed necessary because of the risk of spread of some strains of avian influenza viruses to poultry flocks and to humans. Initially in the UK this included the screening of live-caught waterfowl, shot waterfowl and individual birds found dead (Cromie et al., 2006), and the investigation of mass mortality incidents (MMIs) in wild birds where five or more dead birds were found in the same location and at the same time. In 2010 the surveillance guidelines in the UK changed, with screening of birds found dead on certain designated reserves but no further sampling of trapped or shot wildfowl (Anon 2011a). Investigation of MMIs, however, continued and still continues (Defra 2015). Enhanced statutory screening of wild birds in the UK for avian influenza viruses may be required, following confirmation of the presence of specified avian influenza viruses such as H5N1 in wild birds (Alexander et al., 2010).

Wild bird surveillance to support wildlife rehabilitators

Kirkwood (1993) highlighted a significant increase in the rescue, treatment and rehabilitation of wildlife and predicted that this interest would continue to grow. An awareness of the diseases likely to be found in wild birds would help veterinary surgeons and wildlife rehabilitators arrive at an appropriate differential diagnosis when presented with sick birds. This information could influence treatment strategies, and raise awareness of the implications of subsequent spread of disease to other wildlife in the rehabilitation centre, the dangers of spread to humans, and possible implications if the birds were subsequently released. Kirkwood (1993) warned that “*some dire*

epidemics have occurred in wildlife as a result of ill-considered releases, and the risk of accidentally introducing disease should always be carefully assessed”, although no examples were given. As indicated earlier, wild bird disease surveillance should be a routine part of the rehabilitation process, and the results of this surveillance may help to avert such problems.

Training for those involved in wildlife surveillance

One of the recommendations made by NERC (Osborn et al. 1990) was that there should be appropriate training in wildlife diseases at both undergraduate and postgraduate levels. Knowledge of what diseases are present or have been present in particular species of bird will assist in the training of new generations of veterinary surgeons, biologists and pathologists, and Kirkwood (1993) noted that a suitable information system needed to be developed. Participation in enhanced passive surveillance for the purpose of early-warning surveillance will provide the opportunity to develop this expertise.

Establishment of baseline values

When investigating the causes of increased wild bird mortality, new or re-emerging diseases etc., it is crucial that the “normal” causes of mortality are recognised and understood, so that new or re-emerging diseases can be recognised as such. An awareness of the pathogens, vectors and diseases present at a local, national or international level is also essential when conducting risk assessments as part of wildlife translocations carried out for wildlife management and conservation purposes (Hartley and Gill, 2010). Disease surveillance will help to establish these baseline values.

Monitoring for antimicrobial resistance

Monitoring bacterial isolates from wild birds for evidence of antimicrobial resistance (AMR) may give advance warning of the emergence of new resistant strains of bacteria. The first UK isolation of *Salmonella* Typhimurium definitive phage type (DT) 104 that was resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and

tetracyclines (ACSSuT) was made from a black-headed gull (*Chroicocephalus ridibundus*) in 1984 on the south coast of England (Davies 2001). This penta-resistant strain of *S. Typhimurium* went on to become of major importance in cattle and humans in the UK (Davies 2001). A high prevalence of AMR in bacterial isolates from wildlife was reported by Gilliver et al. (1999), who found that AMR was widespread in coliforms isolated from wild rodents in England. Since then, numerous other studies have demonstrated AMR in bacteria isolated from wildlife or their environment, some of which could impact on human health such as vancomycin-resistant enterococci (VRE) recovered from a black-headed gull in Sweden (Sellin et al., 2000) and from American crows (*Corvus brachyrhynchos*) in the United States (Oravcova et al., 2014), and carbapenemase-producing *Salmonella enterica* subspecies *enterica* serovar Corvallis (*S. Corvallis*) isolated from a black kite (*Milvus migrans*) in Germany (Fischer et al., 2013).

Resistance to the beta-lactam class of antimicrobial (which includes penicillins, cephalosporins and carbapenems) is currently of great concern in human medicine. Resistance to this group is conferred by genes encoding a range of beta-lactamase enzymes, and extended-spectrum beta-lactamases (ESBLs) are of especial significance. There are several different types of ESBL enzymes but currently those of the cefotaximase (CTX-M) family are causing the greatest concern in human medicine, both in hospital situations and in the wider community setting. Guenther et al. (2011) provided a very useful review of ESBL-producing *Escherichia coli* (ESBL-EC) in humans and wildlife, and noted CTX-M producers in gulls from Portugal, the Czech Republic, France, Russia, Sweden and Poland. In Scotland in 2012, CTX-M producing ESBL-EC were recovered from the carcasses of 13 out of 30 (43.3%) dead gulls (*Larus* spp.) screened (SRUC 2015).

1.4.4 Wild bird disease surveillance at Ayr DSC 1993 – 2014 – objectives

The importance of having clearly-defined objectives when carrying out disease surveillance was emphasised by OIE (2011) and Drewe et al. (2012). Disease

surveillance at Ayr DSC evolved over time, as different funders became involved and different types of surveillance were requested (see Chapter 2, Materials and Methods). Defining the population of interest is also important, and for wild bird disease surveillance at Ayr DSC the population of interest was *wild birds found dead or sick in Scotland*. For the purposes of this thesis, the population of interest was further restricted to *wild birds of the orders Passeriformes and Columbiformes found dead or sick in Scotland between 1994 and 2013*. The main objectives of wild bird disease surveillance at Ayr DSC and the subsequent preparation of this thesis can be summarised as follows:

- **Objective 1.** Provision of hazard-specific (targeted) surveillance for specified pathogens (*Salmonella* spp., *Escherichia albertii*, *Mycoplasma* spp., avian influenza viruses, West Nile virus) in the population of interest.
- **Objective 2.** Provision of enhanced passive surveillance to increase awareness of, or add to our understanding of, diseases or pathogens known to be endemic in the population of interest.
- **Objective 3.** Provision of early-warning surveillance to detect exotic, re-emerging or new (emerging) diseases or pathogens in the population of interest.
- **Objective 4.** Provision of enhanced passive surveillance to detect accidental or deliberate harm to wild birds in the population of interest, including poisoning, deaths in birds at garden feeding stations, and losses in rehabilitation centres.
- **Objective 5.** Timely reporting of results and trends to stakeholders, including those funding the surveillance and those submitting the carcasses.
- **Objective 6.** Retrospective review of postmortem findings from wild birds from the population of interest, and assignment of up to three diagnoses based on previously-defined diagnostic criteria.
- **Objective 7.** Preparation of a review of diseases reported from wild birds of the orders Passeriformes and Columbiformes in the UK, collation of results from wild bird surveillance at Ayr DSC between 1994 and 2013, and discussion of the significance and implications of the findings.

- **Objective 8.** Provision of data on the antimicrobial susceptibility or resistance of bacterial isolates from the carcasses of finches and sparrows, and from wild bird faeces from garden feeding stations.
- **Objective 9.** Preparation of a collection of images of pathological lesions, parasites and parasite oocysts or eggs from the population of interest, to be made available to those involved with wildlife disease surveillance, diagnosis or treatment.
- **Objective 10.** Preparation of a summary of descriptions of internal parasites and their oocysts or eggs, to aid in the presumptive identification of internal parasites from the population of interest and to be made available to those involved with wildlife disease surveillance, diagnosis or treatment.

Chapter 2

Materials and Methods

2.1 Introduction

Postmortem examinations were carried out on over 3000 wild birds found in Scotland between January 1994 and December 2013. They were conducted at Ayr Disease Surveillance Centre (Ayr DSC) of what was known as the Scottish Agricultural College Veterinary Services (SACVS), later referred to as SAC Consulting Veterinary Services (SACCVS), part of SRUC (Scotland's Rural College). The staffing at Ayr DSC fluctuated over the years, but when the study ended in 2013 there were four veterinary investigation officers, six scientists and four administrative/support posts. The main functions of Ayr DSC were to provide advisory, diagnostic and surveillance services in farmed livestock, mostly cattle, sheep and poultry, and the work carried out regarding wild birds was a minor part of the overall workload. As a result, there were limitations on time and laboratory resources available for the wild bird work, which influenced the selection of tests carried out. Most (over 98%) of the postmortem examinations were carried out by the author but small numbers were carried out by other members of the veterinary staff at Ayr DSC. A breakdown of the different categories of birds examined is presented in Table 2.1. The findings described in this thesis are restricted to the 2048 carcasses of the orders Passeriformes and Columbiformes, and to pooled faecal samples collected from two garden bird feeding stations in Scotland. The sources and geographic locations of the carcasses are outlined below, as are the sources of funding. The protocols for the necropsies and laboratory work carried out at Ayr DSC are described, and work performed at other locations of SRUC and other organisations are summarised. Statistical analysis of the data was not always appropriate but the tests used are discussed below.

Table 2.1: Categories of wild birds examined at Ayr DSC 1994 to 2013

Category of wild bird	Order	Number of birds examined
Finches, sparrows, buntings, dunnocks and tits	Passeriformes	1278
Blackbirds, thrushes, robins and starlings	Passeriformes	217
Corvids	Passeriformes	292
Warblers; swallows and house martins; nuthatches and tree-creepers; wagtails and pipits; waxwings, wrens and dippers	Passeriformes	49
Pigeons and doves	Columbiformes	212
Falcons, hawks, eagles and owls	Accipitriformes, Strigiformes	234
Ducks, geese and swans	Anseriformes	283
Gulls, gannets, fulmars, shearwaters, auks, waders, cormorants and shags	Charadriiformes, Procellariiformes, Pelecaniformes	455
Others	Various	46
Total		3066

2.2 Sources of carcasses and faeces, and funding of investigations

When examination of wild bird carcasses commenced in 1994, the majority of the carcasses were submitted by a wildlife rehabilitation centre and the investigations were funded by the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD) in order to screen for infectious diseases that could impact on human or livestock health. Between 1995 and 1996, a pharmaceutical company funded a two-year study looking at wild bird diseases, especially those caused by parasites that could affect poultry and game birds, in approximately 400 carcasses submitted directly by members of the public or through a wildlife rehabilitation centre. Increased numbers of deaths in garden birds from 1997, especially in the Grampian region of Scotland, prompted members of the general public to submit many carcasses for necropsy, and SOAEFD funded these investigations because of concerns about salmonellosis that might affect human or livestock health. Additional funding for these investigations was received

from RSPB and another pharmaceutical company. The Scottish Office continued to fund investigations where there was concern for human or livestock health or where agrochemical poisoning was suspected (as part of the Wildlife Incident Investigation Scheme), with carcasses submitted by members of the public, wildlife rehabilitation centres and organisations such as RSPB, Scottish Natural Heritage (SNH) and Scottish Local Authorities (LAs).

The funding for investigations was put on a firmer footing in 2000 when the Dulverton Trust and the Game Conservancy Trust (now the Game and Wildlife Conservation Trust) funded wild bird disease surveillance between May 2000 and September 2003, when over 400 carcasses and nearly 300 samples of pooled faeces from bird tables were examined. A combination of donations from CJ Wildbird Foods Ltd, the SAC Trust Fund, a pharmaceutical company and the Galloway Wildlife Refuge permitted surveillance for salmonellosis to continue to early 2005, with samples of faeces or carcasses collected and submitted by members of the general public or SAC staff. A further boost to funding occurred between May 2005 and March 2008, when participation in the Garden Bird Health initiative (GBHi) added to the already extensive network of members of the public submitting garden bird carcasses from sites in Scotland (Cunningham et al., 2005). The GBHi was funded by a consortium of companies producing food for wild birds, and approximately 400 carcasses were examined at Ayr DSC as part of this initiative.

The Scottish Executive, becoming the Scottish Government in 2007, continued to fund examination of other wild bird carcasses where there was a risk of diseases that could affect humans or livestock, including screening for West Nile virus at certain times of the year, and from October 2005 wild bird surveillance increased again as highly pathogenic avian influenza virus (HPAIV) H5N1 spread into Europe from south-east Asia. Large numbers of wild bird carcasses were collected and submitted by the Field Service of the Animal Health Veterinary Laboratories Agency (AHVLA, now Animal and Plant Health Agency [APHA]) after HPAIV H5N1 was found in a wild whooper

swan washed up in Scotland at the end of March 2006 (Alexander et al., 2010), before reducing again in October 2006. In April 2007 wild bird disease surveillance was made a specifically funded advisory activity of the Scottish Government's Public Good Veterinary and Advisory Services (VAS) programme, and was still in place at the end of 2013 when this 20-year study concluded. Under the VAS programme wild bird carcasses could be examined as part of mass mortality incidents (five or more wild birds found dead at one time at the same place), if diseases that could affect humans or livestock were suspected, if there was public concern, or if agrochemical poisoning was suspected.

2.3 Locations of carcasses

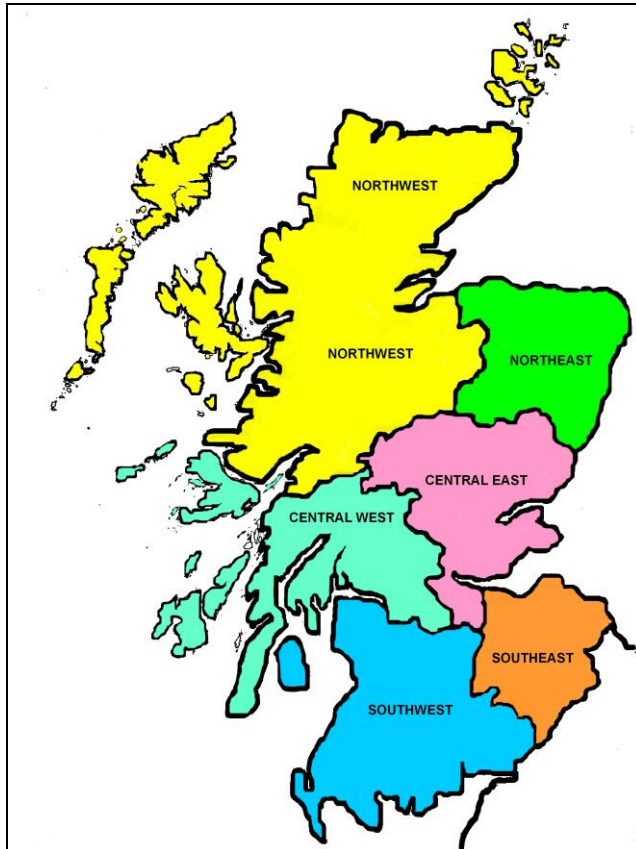
Only carcasses found in Scotland were included in this study. Scotland was divided into six different areas based on the 32 Scottish Local Authority (LA) areas, and each carcass was assigned to an area depending on the post code or Ordnance Survey grid reference in which the carcass was found. If this information was not available or uncertain, the location was described as "not recorded". The six areas were as follows:

- Northwest: LA areas of Highland, Orkney Islands, Shetland Islands, Western Isles
- Northeast: LA areas of Aberdeenshire, Aberdeen City, Moray
- Central West: LA areas of Argyll and Bute, Glasgow City, North Lanarkshire, East Dunbartonshire, West Dunbartonshire, Stirling
- Central East: LA areas of Perth and Kinross, Angus, Clackmannanshire, Fife, Falkirk, West Lothian, Dundee City
- Southwest: LA areas of North Ayrshire, East Ayrshire, South Ayrshire, Renfrewshire, East Renfrewshire, Inverclyde, South Lanarkshire, Dumfries and Galloway
- Southeast: LA areas of Midlothian, East Lothian, Edinburgh City, Scottish Borders

These six areas are illustrated in Figure 2.1. For the purposes of data analysis, the northwest and northeast areas were combined to form region 1 (north of Scotland), and central west, central east, the southwest and southeast areas formed region 2 (south of Scotland). When plotting the spread of trichomonosis (Chapter 6), three sub-regions

were considered – southwest/southeast, central west/central east, and northwest/northeast.

Figure 2.1: Division of Scotland into six areas



2.4 Work carried out at Ayr DSC

2.4.1 Necropsy protocol (gross examination)

During the twenty-year duration of the study several different necropsy protocols were used, some of which are shown in Appendix IX. Due to limited resources, the protocol followed was customised for individual necropsies, dependent on the species or group of bird, the project under which the carcass was submitted, the degree of autolysis, and the resources available. In general terms each carcass was identified to species level, examined externally, weighed and immersed in disinfectant. The skin was reflected from

the breast, abdomen and legs, and body condition assessed on fat coverage and prominence of the sternum. The head was skinned and in larger birds the infraorbital sinuses examined. Scissors were used to open the oesophagus and crop, and in larger birds the larynx and trachea. In some studies the entire trachea was removed to facilitate examination for parasites. The abdomen was opened below the sternum and the viscera exposed by blunt dissection through the airsacs. The thoracic viscera were visualised by cutting through the ribs and bones of the shoulder using scissors or bone shears as appropriate. Heart, lungs and airsacs, liver, spleen and kidneys were removed or examined *in situ*. The proventriculus and gizzard were routinely opened and inspected and in larger birds the intestines were opened at several locations. Where possible, sex and approximate age were determined by examination of the plumage, gonads, and size of the bursa of Fabricius. In some projects the brain was removed by parasagittal section of the skull, and joints and muscles were incised if grossly abnormal. For human health and safety, carcasses were examined in a Class 1 microbiological safety cabinet.

2.4.2 Wet preparations

In some birds, wet preparations were made from a variety of sites and examined microscopically at x100 and x400. Such locations included oropharynx, oesophagus, proventriculus, and different levels of intestine. The wet preparations were made by placing two separate drops of saline on to a 76mm x 22mm glass slide, scraping the mucosa or surface of the organ with a 32mm x 22mm coverslip, transferring a small portion of material to the saline, and placing the coverslip on top. The unstained wet preparations were examined for the presence of helminths or their eggs, coccidial oocysts, motile protozoa such as trichomonads and *Spiroplasma* spp., and avian gastric yeasts (“megabacteria”). Resource restrictions meant that wet preparations were not carried out in every bird, and the decision to carry out this examination and the sites selected was dependent on numerous factors including the species of bird, nature of the project being funded, degree of autolysis, postmortem findings and time availability. Almost all of the wet preparations were examined by T. Pennycott.

2.4.3 Bacteriology

Unless specified to the contrary, all bacteriology was carried out by the scientific team at Ayr DSC. The extent of the bacteriology depended on the bird species, postmortem findings, project under which the bird was submitted and degree of autolysis. In some birds no bacteriology was performed. A frequent approach was to inoculate samples from liver and intestine on to Columbia agar supplemented with 5% sheep blood (Oxoid), on to MacConkey agar (Oxoid), and pooling these tissues into selenite broth (Oxoid). Depending on the postmortem findings, additional organs were cultured as appropriate. The cultures were incubated aerobically in 10% CO₂ for 18-24 hours at 37°C. The selenite broth was then subcultured on to deoxycholate citrate lactose sucrose (DCLS) agar (Difco) or brilliant green agar (BGA, Oxoid) and incubated aerobically for a further 18-24 hours at 37°C. The identity of bacterial colonies was established using standard bacteriology techniques including colonial morphology, primary characterisation tests such as Gram stain, catalase, oxidase, indole and coagulase reactions, and if necessary further characterisation was achieved using commercial tests such as API and RAPIDEC Staph strips (Biomérieux). Where bacterial colonies suggestive of *Salmonella* sp. were detected, slide agglutination tests were carried out with polyvalent O and H antisera (Mast Laboratories), and additional biochemical tests performed using API kits. Isolates considered to be *Salmonella* sp. were then sent to the Scottish Salmonella Reference Laboratory (SSRL) for microtitre serotyping using somatic and flagellar antigens and final phage typing (Anderson et al., 1978). Some non-lactose fermenting organisms that gave negative reactions to O and H antisera had API-20E profiles of 5144102 or 4144102, and were examined at SAC Inverness by a slide agglutination test against *Escherichia coli* O86:K61 antigen (Foster et al., 1998). Faecal samples from bird tables were examined in a similar fashion, with mixing of the sample using a sterile swab followed by plating out on MacConkey agar and into selenite broth. Additional culture plates or conditions were requested in certain circumstances, for example Sabouraud's dextrose agar was used if fungal, including yeast, infections were suspected, or anaerobic cultures if the involvement of organisms such as *Clostridium perfringens* was suspected. On occasions impression smears from postmortem tissues

were stained using Gram, Ziehl-Neelsen or modified Ziehl-Neelsen stains to look for *Clostridium* spp., *Mycobacterium* spp. and *Chlamydia* spp. respectively, using standard techniques. When considered appropriate, antimicrobial susceptibility tests were carried out by the disc diffusion method (BSAC 2013) as described in Appendix IV.

2.4.4 Parasitology

Internal parasites were observed at necropsy either grossly or on microscopic examination of wet preparations (2.4.2) from a variety of sites. In a small number of submissions, intestinal contents or faeces were prepared for further examination by the scientific team at Ayr DSC using an abbreviated non-quantitative McMaster technique: the sample was added to a beaker containing glass beads, up to 45ml of water added and shaken well, the sample was passed through a 150µm sieve into a centrifuge tube, centrifuged for 10 minutes at 1200 revolutions per minute, the supernatant poured off and the pellet re-suspended in saline for microscopy. On some occasions where trichomonosis was suspected, oesophagus or crop was cultured in Bushby's Trichomonas Medium No. 2 (Oxoid) for 5 days at 30°C, followed by microscopy. Most external parasites were visible to the naked eye at necropsy, but on occasion scab material or mites were placed in 20% potassium hydroxide (KOH) for 15-30 minutes to aid detection or identification. When considered appropriate, specimens of helminths or external parasites were retained in alcohol or formol saline. Images of selected worm eggs or coccidial oocysts were captured using a 5 megapixel digital camera (Spot Idea) attached to an Olympus microscope, and the dimensions calculated by Spot Basic computer software (Spot Imaging Systems, a division of Diagnostic Instruments Inc., Michigan, USA). Prior to acquiring this software, measurements of eggs were obtained using an in-house computer-based diagnostic system.

Identification of parasites or their eggs in most cases was made to group or genus level rather than species level. Further details of the criteria used for the presumptive identification of the internal parasites are listed in Appendix III. Most of the parasites

were identified within the above constraints by T. Pennycott, but a small number were identified by Mrs Eileen Harris of the Natural History Museum, London.

Microscopy of stained blood smears for the presence of haematozoa was not carried out due to limitations in time resources and expertise.

2.4.5 Histopathology

As part of the necropsy protocol for the GBHi, small portions of brain, liver, kidney, lung, heart and spleen were, if available, routinely retained in 10% buffered formol saline for possible future examination. Selected tissues were similarly collected from other necropsies if required to establish a diagnosis, subject to resource constraints and degree of autolysis. Histological sections were produced and stained with haematoxylin and eosin (H&E) using standard techniques by scientific staff at SAC Edinburgh or AHVLA/APHA Lasswade. Other stains such as Gram, Periodic Acid Schiff (PAS) and Congo Red were sometimes requested, and immunohistochemical staining for specific organisms was occasionally carried out by AHVLA/APHA Lasswade or Moredun Research Institute (MRI). Histopathological interpretation of the sections was performed by T. Pennycott or histopathologists at SRUC, AHVLA, MRI or Abbey Veterinary Services, Newton Abbot. Specialised histopathology of the eyes of nestling choughs (*Pyrrhocorax pyrrhocorax*) was carried out by Dr John Mould of Eye Veterinary Clinic, Leominster, Herefordshire.

2.4.6 Statistical analysis

Limited statistical evaluation of the data collected was possible due to the nature of the data. Descriptive statistics (mean, mode, median, standard deviation and sample variance) were obtained for the measurements of coccidial oocysts and helminth eggs using Microsoft Excel 2010, and the dimensions expressed as mean \pm 2 standard deviations in Appendix III.

When comparing the proportions of male and female birds affected by different conditions in Chapter 7, a two-sample binomial test was employed, using GenStat

Release 15.1 (2012), VSN International Ltd, Hemel Hempstead, HP2 4TP. The null hypothesis was that the observed sex ratio did not differ from an expected ratio of 50:50, and the probability p calculated using a two-tailed test and 95% confidence interval. P values <0.05 were considered to be statistically significant.

A two-tailed Fisher's exact test [GenStat Release 15.1 (2012)] was used to compare categorical data from different groups presented in a 2x2 contingency table, for example different phage types of *Salmonella* spp. in different regions of Scotland, or the isolation/non-isolation of *Escherichia albertii* from finches and from non-finches. The null hypothesis was that there was no association between row variables and column variables, and the null hypothesis was rejected if $p < 0.05$. Fisher's exact test was used in preference to Pearson's chi-square test because some frequencies were very small or zero.

Tables listing the conditions diagnosed in different categories of birds included 95% confidence intervals in addition to number of birds and percentage of birds with each condition. These confidence intervals were also used in the text when describing or discussing the results. Confidence intervals were included to take into account the uncertainty caused by small sample sizes, providing an estimate of the margin of sampling error. An on-line statistical calculator <http://vassarstats.net/prop1.html> was used to calculate the confidence interval for binomial proportions, using Wilson's procedure with correction for continuity.

2.5 Work carried out at other SRUC locations or by other organisations

In addition to the work described above, more specialised testing was required in some cases to further identify potential pathogens, establish a diagnosis or screen for specified pathogens. The tests and testing laboratories are listed below.

2.5.1 Microbiology

- *Salmonella* spp. serotyping and phage typing by SSRL, Stobhill Hospital, Glasgow G21 3UW.

- Serotyping/phage typing of isolates of *Campylobacter jejuni* and *Yersinia enterocolitica* by Public Health Laboratory Service, Colindale Avenue, London NW9 5HT.
- Culture and identification of *Mycoplasma* spp. by Department of Veterinary Pathology, Liverpool University, Neston CH64 7TE.
- Culture for *Mycobacterium avium* by SACCVS Perth, now 5 Bertha Park View, Perth, PH1 3FZ; MRI, Pentlands Science Park, Penicuik, EH26 0PZ; and Scottish Mycobacteria Reference Laboratory, Edinburgh Royal Infirmary, Old Dalkeith Road, Edinburgh EH16 4SU.
- Capsular typing of *Pasteurella multocida* by MRI
- Screening for *E. coli* O157 and associated virulence genes by SACCVS Inverness, Drummondhill, Inverness IV2 4JZ and by AHVLA/APHA, Weybridge, KT15 3NB.

2.5.2 Molecular diagnostic tests

- Detection of *Trichomonas gallinae* by polymerase chain reaction (PCR) by Institute of Zoology (IoZ), Regent's Park, London NW1 4RY followed if required by nucleotide sequencing and comparison with Genbank data by Beckman Coulter Genomics, High Wycombe HP11 1JU.
- PCR for *Ornithobacterium rhinotracheale* by Molecular Diagnostic Testing, The Grove, Craven Arms, Shropshire SY7 8DA.
- PCR for *Coxiella burnetti* by AHVLA/APHA, Penrith CA11 9RR.
- PCR for avian metapneumoviruses A, B and C by AHVLA/APHA, Penrith
- PCR for *Chlamydia* spp. by Biobest Laboratories Ltd, The Edinburgh Technopole, Penicuik EH26 0PY and by AHVLA/APHA, Weybridge, KT15 3NB.
- PCR for pigeon circovirus by Biobest Laboratories Ltd
- PCR for avian pox by IoZ
- PCR for *Avibacterium paragallinarum* by GD Animal Health Services, 7400 AA Deventer, The Netherlands.

- PCR (IDDEX and Adiavet MycoAV kits) for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by Department of Veterinary Pathology, Liverpool University
- Examination of *Mycoplasma gallisepticum* isolates by Random Amplified Polymorphic DNA analysis by Department of Veterinary Pathology, Liverpool University
- PCR for *Pasteurella multocida* by IoZ
- PCR for mycobacterial insertion sequences IS900 and IS901 by MRI
- PCR for *Borrelia burgdorferi* sensu lato by Pinmoore Animal Laboratory Services Ltd, Tarporley, Cheshire CW6 0EG.
- PCR for *Staphylococcus aureus mecA* genes (standard and divergent homologues) by Scottish MRSA Reference Laboratory, Glasgow G21 3UW.

2.5.3 Toxicology and biochemistry

- Toxicology (multi-residue analysis for carbamate, organochlorine, organophosphorus and pyrethroid compounds, and a second multi-residue analysis for anticoagulant rodenticides, supplemented when appropriate by compound-specific analytical methods for chloralose, metaldehyde, paraquat, strychnine, and other compounds) carried out under the Wildlife Incident Investigation Scheme by SASA (Science and Advice for Scottish Agriculture, formerly the Scottish Agricultural Science Agency), Roddinglaw Road, Edinburgh EH12 9FJ.
- Tissue ethanol levels by the Department of Forensic Medicine and Science, University of Glasgow, Glasgow G12 8QQ.

2.5.4 Virology

- Electron microscopy of tissues (for papilloma and pox viruses) by MRI
- Screening for avian influenza virus (real-time quantitative PCR or reverse transcriptase-PCR [RT-PCR] for avian influenza m gene, and sometimes passage in embryonated chicken eggs) by AHVLA/APHA, Weybridge

- Screening for West Nile virus (RT-PCR, and sometimes passage in embryonated chicken eggs and Vero cell cultures) by AHVLA/APHA, Weybridge
- Virus isolation in Vero cells, embryonated chicken eggs, chick embryo liver cells or baby hamster kidney cells by AHVLA/APHA, Weybridge
- Virus isolation in pig kidney cells and day-old Portan mice by MRI

2.5.5 Serology

- Serology (haemagglutination inhibition test) for louping-ill by MRI
- Serology (haemagglutination inhibition tests) for infectious bronchitis, egg drop syndrome 76, paramyxovirus-1 and paramyxovirus-3 viruses by AHVLA/APHA, Weybridge
- Serology (agar gel precipitin tests) for avian adenoviruses and avian influenza viruses by AHVLA/APHA, Weybridge

2.5.6 Additional histopathology

- Immunohistochemical labelling of fixed tissues for *Chlamydia* spp. and for *Pasteurella multocida* by MRI
- Immunohistochemical labelling of fixed tissues for *Mycoplasma gallisepticum* by AHVLA/APHA Lasswade
- Immunoperoxidase staining of fixed tissues for *Toxoplasma* spp. and *Neospora* spp. by AHVLA/APHA Lasswade

2.5.7 Parasitology

- Identification of helminths by Parasitic Worms Division (now Parasites & Vectors Division), Natural History Museum, Cromwell Road, London SW7 5BD.
- Identification of ticks from passerines by Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G61 1QH.
- Identification of haemoparasites in histopathological sections by Mike Peirce, MP International Consultancy, Wokingham, Berkshire RG41 3AZ

2.6 Data recording, reporting and retrospective analysis

Each carcase was allocated a unique submission reference number and the details regarding species of bird, submitter, funder etc. recorded electronically. Initially the submission form was printed and the appropriate necropsy form attached, but during the course of the surveillance period a Laboratory Information Management System (LIMS) was developed in which the necropsy form was integral. Postmortem findings were manually added to the appropriate necropsy form and stored for a minimum of seven years. Initially laboratory findings were also manually entered on the necropsy form, but as the LIMS developed, laboratory findings were electronically entered into LIMS. A report summarising the postmortem findings, laboratory results if appropriate and diagnosis as to the cause of death was sent to members of the public or wildlife rehabilitation centres submitting carcases. Summaries were also sent to major funders such as the Dulverton Trust, the Garden Bird Health initiative and the Scottish Government, and significant findings summarised in the monthly reports of SACCVS in the Veterinary Record.

A summary spreadsheet was created in Microsoft Excel near the start of the surveillance period and appropriate data regularly entered from the necropsy forms and LIMS. The numbers of data columns in the spreadsheet were periodically increased to aid data analysis for the purposes of this thesis. Personal information about those submitting carcases was removed from the spreadsheet to ensure compliance with data protection legislation.

Chapter 3

Diseases (excluding salmonellosis, *Escherichia albertii* bacteraemia and trichomonosis) of UK finches, sparrows, buntings, dunnocks and tits

3.1 Introduction

This chapter considers disease conditions (excluding salmonellosis, *Escherichia albertii* bacteraemia and trichomonosis) found in some of the commonest small garden birds, including finches (Families Carduelidae and Fringillidae), sparrows (Family Passeridae), buntings (Family Emberizidae), accentors (Family Prunellidae) and tits (Families Paridae and Aegithalidae). Depending on the species, these birds feed primarily on insects, spiders and caterpillars, seeds, nuts and grains. Starlings, robins, blackbirds and thrushes, whose diet relies more heavily on soil invertebrates such as earthworms and leatherjackets, and in some species fruit, are addressed in Chapter 8. Salmonellosis in finches, sparrows, buntings, dunnocks and tits is covered in Chapter 4, *E. albertii* bacteraemia in Chapter 5, trichomonosis in Chapter 6, and interactions between these three diseases are discussed in Chapter 7. The Latin names of the UK birds discussed are listed in Appendix I.

Musgrove et al. (2013) estimated that there are over 10 million breeding pairs of finches in the UK, the commonest of which found in gardens are the chaffinch, greenfinch, goldfinch, bullfinch (*Pyrrhula pyrrhula*), siskin and lesser redpoll (*Carduelis cabaret*). In winter large numbers of chaffinches and bramblings (*Fringilla montifringilla*) come to the UK from Fennoscandia and northern Europe, and in some years there are large irruptions of siskins into the UK from these areas. There are approximately 5 million breeding pairs of house sparrows in the UK, and much smaller numbers (200,000) of breeding pairs of tree sparrows (*Passer montanus*) (Musgrove et al., 2013). Accentors in the UK are represented by the dunnock or hedge accentor (*Prunella modularis*, once referred to as the hedge sparrow), of which there are some 2.5 million breeding pairs in

the UK, and there are 7.5 million breeding pairs of tits, mostly blue tits, great tits, coal tits (*Parus ater*) and long-tailed tits (*Aegithalos caedatus*) (Musgrove et al., 2013). Buntings such as yellowhammers (*Emberiza citrinella*) and reed buntings (*E. schoeniclus*) are seen less frequently in gardens but have a combined breeding population of nearly one million pairs (Musgrove et al., 2013) and are included in this chapter because of their similarity from a dietary and disease perspective to finches and sparrows. The house sparrow, tree sparrow, lesser redpoll and yellowhammer are red-listed as being of conservation concern in the UK (Hayhow et al., 2014).

3.2 Review of diseases (excluding salmonellosis, *E. albertii* bacteraemia and trichomonosis) found in UK finches, sparrows, buntings, dunnocks and tits

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

3.2.1 Trauma

The commonest cause of death of garden birds that have been rung and subsequently found dead near human habitation is trauma, either human-related (e.g. window collisions and road traffic accidents) or by domestic predators, especially cats. For example, when the causes of death were known, these two categories accounted for 81% of rung greenfinches found dead (Main 2002), 79% of chaffinches (Norman 2002), 77% of house sparrows (Summers-Smith and Thomas, 2002), 86% of dunnocks (Hartley 2002) and 83% of blue tits (Gosler 2002).

3.2.2 Pasteurellosis

Secondary pasteurellosis in garden birds that have been caught by cats is well recognised (Macdonald 1981). Stocker (2000) noted that birds initially surviving a cat attack frequently died within 48 hours due to secondary *Pasteurella multocida* septicaemia originating from the oral cavity of the cat.

3.2.3 Infection with *Suttonella ornithocola*

Multiple mortality incidents associated with the Gram-negative bacterium *Suttonella ornithocola*, involving blue tits, coal tits, great tits and long-tailed tits, occurred in England and Wales in 1996 (Kirkwood et al., 2006). Clinical signs were non-specific and postmortem findings usually unhelpful, although some birds had congested lungs. Histopathology was not carried out but an organism identified as *S. ornithocola* was subsequently isolated from the lung of four birds. Further details about the characteristics of this bacterium were presented by Foster et al. (2005). The significance of *S. ornithocola* was initially unclear but Lawson* et al. (2011b) reported a further six incidents in tits from which *S. ornithocola* was isolated and showed that in some birds the organisms were associated with acute necrotising pneumonia. Cases occurred between March and May (Kirkwood et al., 2006) and late January to April (Lawson* et al., 2011b), suggesting a seasonal pattern.

3.2.4 Infection with *Chlamydia (Chlamydophila) psittaci*

Infection of wild birds with *Chlamydia (Chlamydophila) psittaci* has historically been more commonly associated with wild pigeons and doves in the UK (Gough and Bevan, 1983; Sharples and Baines, 2009) but has also been reported in small garden birds. Pennycott* et al. (2009) described the demonstration of *C. psittaci* in blue tits (significance unknown) and a chaffinch – the latter bird had lesions of presumed trichomonosis of the oesophagus but also had a fibrino-necrotic airsacculitis. *C. psittaci* was also detected in a robin found dead at the same location as the chaffinch and which had splenitis, hepatitis and airsacculitis, and deaths in robins from chlamydiosis have been reported elsewhere (Simpson and Bevan, 1989). Mortality incidents in England involving robins, tits or dunnocks were investigated by Colvile et al. (2012) and *C. psittaci* was detected by PCR in four dunnocks, three robins and three great tits. This study was expanded by Beckmann et al. (2014a) who detected *C. psittaci* in a further 8 dunnocks (8/8 tested), 7 out of 12 great tits tested, 3 out of 4 blue tits, 2 out of 3 collared doves and 1 out of 4 robins. Postmortem lesions frequently included splenomegaly, hepatomegaly, fibrinous serositis, and poor body condition. On the basis of

histopathology and immunohistochemistry specific for *Chlamydia* spp., these authors concluded that in at least 10 birds the organism was contributing to the pathology observed. Concurrent conditions including trauma, pox and trichomonosis were seen in many of the *Chlamydia*-positive birds. Genotype A was demonstrated in 21 passerines (dunnocks, robins, great tits and blue tits) and Genotype E was confirmed in 2 collared doves (Beckmann et al., 2014a). Wild garden birds have been implicated as being a source of *C. psittaci* infection in humans in Australia (Telfer et al., 2005) and Sweden (Rehn et al., 2013), and Genotype A is the commonest genotype identified in humans (Beckmann et al., 2014a). Infection of small garden birds, especially dunnocks, robins and tits, with *C. psittaci* may be more widespread than previously thought, and Pennycott* et al. (2009) commented that hygiene measures were essential at wildlife rehabilitation centres and by members of the public to minimise the risk of spread of this organism between wild birds or from wild birds to humans.

3.2.5 Infection with *Mycobacterium avium* (avian tuberculosis) and *Yersinia pseudotuberculosis*

In a review of avian tuberculosis in birds, Wilson (1960) noted that in some flocks of house sparrows feeding alongside pigs in Denmark, up to 40% of the birds were infected with *Mycobacterium avium*, but provided no evidence for its occurrence in sparrows or other small garden birds in the UK. Yersiniosis (pseudotuberculosis), caused by *Yersinia pseudotuberculosis*, has been recorded sporadically in small garden birds in the UK, and Mair (1973) lists isolations of *Y. pseudotuberculosis* from great tit, greenfinch, dunnock and tree sparrow. Postmortem lesions typically include enlargement of the liver and spleen, frequently with multiple pale foci, nodules in the duodenum and intestine, and sometimes a purulent arthritis. *Y. pseudotuberculosis* can also cause disease in humans, usually causing a relatively mild enteric disease, but occasionally resulting in more severe signs that clinically resemble acute appendicitis (Mair 1973). Human infections are most often acquired following direct or indirect contact with pet or wild mammals or birds (Mair 1973).

3.2.6 Avian pox, *Cnemidocoptes* sp. mites, cutaneous papillomas, mycotic skin infections

Certain species of small garden birds are susceptible to conditions that cause gross abnormalities of the feet, legs, skin and beak. Avian pox is seen most often in house sparrows, dunnocks and great tits, scaly leg caused by *Cnemidocoptes* sp. mites has been recorded mostly in chaffinches, and cutaneous papillomas are seen in chaffinches and bramblings. Mixed infections may also occur but could be overlooked unless comprehensive testing was carried out.

Avipoxvirus infection can result in nodules around the eyes, at the commissures of the beak, on the skin and on the legs and feet. Historically house sparrows and dunnocks have been the small garden birds most frequently affected (Blackmore and Keymer, 1969). However, multiple cases of avian pox occurred in great tits in Sweden in 2003 and in Austria, Hungary, Germany, Slovakia and the Czech Republic from 2005 (Literak et al., 2010). In 2006 the disease appeared in a great tit in England (Lawson et al., 2012a), and since then the condition has spread to multiple other sites, initially in south-east England but gradually moving north. Although most cases have been in great tits, there have also been unconfirmed incidents affecting other tit species (Lawson et al., 2012a). The lesions described in tits tended to be larger than those described in house sparrows and dunnocks, sometimes approaching 2 cm in diameter, and on cut surface often had a yellow caseous core. The nodules were usually on the head or body, unlike pox in dunnocks which more often affects the legs and feet. Most incidents in great tits were reported in August and September, possibly reflecting increased susceptibility of current year birds (Lawson et al., 2012a). Genetic sequencing showed that the avipoxvirus from great tits in Sweden, central Europe and the UK had 100% similarity, and were also identical to poxvirus from dunnocks and very similar to poxvirus from house sparrows. Poxvirus from woodpigeons and a starling were, however, different (Lawson et al., 2012a). Although adversely affecting individual birds, Lachish et al. (2012) concluded that avian pox was unlikely to have a significant effect at a population level.

Burrowing mites of the genus *Cnemidocoptes* (also referred to as *Knemidocoptes*) can cause lesions on the feet, face or skin of a range of avian species, including wild finches. In the UK most cases have been reported in chaffinches (Macdonald 1962a; Macdonald and Gush, 1975; Anon 2003; Anon 2012a), but a few cases have been described in greenfinches and bullfinches (Macdonald and Gush, 1983). Examination of ringed birds of known ages, sometimes captured on several occasions, showed that birds were often around 20-24 months when lesions were first noted, and that some birds could be affected for three to 15 months (Macdonald and Gush, 1975). These authors also reported lesions in two nest-mates when they were aged nearly two years, and they suggested that infection could be acquired in the nest but with a long incubation period. The causal mite is considered to be *C. jamaicensis* (Macdonald and Gush, 1983), although Dabert et al. (2013) queried whether *C. jamaicensis* was actually a complex of multiple species of mite infecting a wide range of avian hosts.

Cutaneous papillomas involving the digits and lower legs of wild UK chaffinches were first noted in 1959 (Jennings 1959) and more fully described by Keymer and Blackmore (1964) and Blackmore and Keymer (1969). Affected birds typically have a large, rough, irregular mass surrounding and enclosing their toes, sometimes extending up the lower leg. The growths may reach several centimetres in size and affect one or both legs. Mostly wild chaffinches (and to a lesser extent bramblings) are affected, but the condition has also been seen in aviary-bred chaffinches and greenfinches (Blackmore and Keymer 1969; Sironi and Gallazzi, 1992). Histopathology demonstrates typical papillomatous changes, hyperkeratosis and acanthosis (Blackmore and Keymer, 1969), and large numbers of papillomavirus-like particles may be seen on electron microscopy (Pennycott* 2003). The causal agent has been named *Fringilla papillomavirus* (Erdelyi 2012) and is thought to be pathogenic only in finches. Affected birds may have difficulty in walking, and in severe cases may be unable to find food and die from starvation or predation, but the lesions may regress spontaneously in mildly affected birds (Erdelyi 2012). Less commonly, lesions may develop on the beak, causing proliferative lesions

that can extend into the oral cavity (Literak et al., 2005). Mixed infections have been reported; Macdonald and Gush (1975) reported a combination of cnemidoptotic mange and papillomavirus lesions in a chaffinch, with lesions of mange preceding the development of the papilloma, and Literak et al. (2005) described concurrent infection with papillomavirus and *C. jamaicensis* in a chaffinch in the Czech Republic. Perez-Tris et al. (2011), using a multiplex PCR, detected both avian poxvirus and avian papillomavirus in archived material from a chaffinch from the Balearic Islands. Macdonald and Gush (1975) considered that lesions of cnemidoptotic mange could be readily differentiated from papillomas, the former presenting with “pumice stone-like” lesions on the tarsus and the latter showing cauliflower-like growths around the foot joint.

Feather loss and thickening/crusting of the skin of two house sparrows, two dunnocks, a blue tit and a bullfinch was associated with unidentified fungal infections (Blackmore and Keymer, 1969). Affected areas included the head, ventral neck, wing and inner thigh, and histopathology showed hyperkeratosis and fungal hyphae infiltrating the keratinised layers and feather follicles. Attempts to isolate pathogenic fungi were unsuccessful. These authors also described feather loss in small garden birds in which no further investigations were possible, but in which fungal involvement was suspected.

3.2.7 Ticks and tick-borne spirochaetes

The commonest tick-borne zoonosis of humans in North America and Europe is Lyme disease, caused by the spirochaete *Borrelia burgdorferi* sensu lato and transmitted by the tick *Ixodes ricinus* (James et al., 2011). In Europe small mammals are the most important reservoir of *B. burgdorferi* s.l., but James et al. (2011) showed that wild passerines in Scotland can also carry *I. ricinus* and *B. burgdorferi* s.l. Tick larvae and nymphs (all identified as *I. ricinus*) were frequently found in small passerines such as dunnocks, tits and finches, although the highest burdens of ticks were recorded in blackbirds (James et al., 2011). Some of the tick larvae and nymphs were positive by PCR for *Borrelia garinii*, one of the genotypes of *Borrelia* causing Lyme disease in

humans, but the authors commented that more work was needed to determine the threat posed to humans. Sometimes ticks can provoke a severe reaction in birds, and Macdonald (1962b) described severe bruising under the skin of the head of a house sparrow that had three adult *I. ricinus* ticks attached. This was believed to be the result of efforts of the bird to remove the ticks, but a further case in a house sparrow (Anon 2010c) was considered to be a response to toxic substances present in tick saliva. In the latter bird, the presence of two (unidentified) ticks on the forehead of the bird was associated with extensive haemorrhage, necrosis and oedema over the dorsal skull. A similar tick-related syndrome in captive and wild birds infested with the tick *Ixodes frontalis* has been described by Monks et al. (2006), and appears to be especially common in collared doves (Anon 2010c). The possible role of toxic substances in tick saliva remains unsubstantiated, and the pathogenesis of this tick-related syndrome is still unclear.

3.2.8 Haematozoa

Blood parasites of the genera *Plasmodium* (“avian malaria”) and *Haemoproteus* can frequently be detected in wild birds, including small garden birds in the UK, but a comprehensive review of the literature by Bennett et al. (1993) suggested that such infections were not associated with mortality. Nevertheless, Lachish et al. (2011), working with blue tits, concluded that some species of *Plasmodium* had a detrimental effect on host fitness and survivability. In another study, using a nested PCR on archived samples of frozen liver and/or spleen, haemoparasites were detected in 48 out of 248 garden birds tested, including tits and dunnocks (Anon 2013b).

3.2.9 Internal parasites – isosporoid coccidia

Two genera of coccidia, *Isospora* and *Atoxoplasma*, are known to commonly infect passerines. Both produce faecal oocysts with thick walls which when sporulated have two sporocysts, making differentiation difficult and contentious. Historically those completing their entire life cycle in the epithelium of the intestine have been considered to be *Isospora* spp. and those in which merozoites have been identified in blood or

tissues have been described as *Atoxoplasma* spp. Molecular characterisation of these coccidia now throws doubts upon the validity of this approach and Schrenzel et al. (2005) proposed that all should be described as “isosporoid coccidia”. The earlier work cited below, however, refers to *Isospora* spp. and *Atoxoplasma* spp.

Jennings and Soulsby (1957) and Jennings (1959) considered that coccidiosis, most often duodenal, caused or contributed to the deaths of house sparrows, blue tits, chaffinches and a greenfinch, but no further descriptions were given. Coccidial oocysts, most likely *Isospora* spp., were found by Keymer et al. (1962) in 68 of 190 house sparrows but only in one bird was considered to be the cause of death, and Anwar (1966) described the morphology of *Isospora* spp. in 70 wild birds (house sparrows, greenfinches and chaffinches) caught in England – oocysts were found in every bird examined. Coccidial oocysts provisionally identified as *Isospora* sp. or *Atoxoplasma* sp. were believed to be incidental findings in chaffinches, greenfinches, goldfinches and bullfinches that died in Scotland from a range of conditions (Pennycott* et al., 1998). It would appear, therefore, that such oocysts are commonly found in wild finches and sparrows in the UK but in normal circumstances rarely cause problems. However, deaths from isosporoid coccidia were described in young ciril buntings (*Emberiza cirilus*) caught from the wild in England and housed as part of a capture/translocation exercise to help conserve an endangered population (McGill et al., 2010). The birds were removed from the wild when aged approximately six days but subsequently died when 3-10 weeks old. Deaths from isosporoid coccidia identified as *Atoxoplasma* sp. have also been described in young captive bullfinches (McNamee et al., 1995) and young captive greenfinches (Cooper et al., 1989). Thus, although seldom a problem in free-living passerines, isosporoid coccidia can cause mortality in captive birds, whether initially hatched in the wild or aviary-bred.

3.2.10 Internal parasites - helminths

Helminths have been less commonly reported in small garden birds than coccidia, but hairworms were found in a house sparrow and tapeworms (*Anochotaenia globata*) in a

chaffinch (Jennings and Soulsby, 1957). Campbell (1935) found gapeworms (*Syngamus trachea*) in 3/91 house sparrows captured at one location, but not in any of 126 house sparrows from a different site. The three infected birds (one adult, two immatures) each had one pair of gapeworms.

3.2.11 Adverse environmental conditions

During periods of prolonged cold weather, deaths in passerines such as small garden birds may occur, when the birds cannot find sufficient food (Best 2003), although Newton (1972) makes no reference to mortality in finches associated with poor weather. Ash and Sharpe (1964) commented that large numbers of birds died during a prolonged cold spell lasting over 70 days in the winter of 1962-1963, and summarised the postmortem findings of over 200 birds including four dunnocks, eight finches and a house sparrow. However, blackbirds and thrushes were represented in greater numbers. Insufficient food during the winter months due to changes in farming practice was one of the proposed explanations for a significant reduction in house sparrow numbers in rural parts of GB (Hole et al., 2002).

3.2.12 Poisons and toxins

Thirty-two house sparrows were found dead in a factory in England that produced specialised metal castings, and representative birds had congested haemorrhagic lungs (Baker 1977). It later transpired that large quantities of chlorine gas had been accidentally released. Similar postmortem findings were noted in house sparrows in an incident in England in 1987, in which 80-100 birds died (Pennycott and Middleton, 1997): exposure to polytetrafluoroethylene (PTFE) was strongly suspected. Newton (1972) commented that consuming grain coated with organochlorine compounds resulted in a fall in the chaffinch population in GB in the 1960s. The increased provision of peanuts to garden birds raised the possibility of exposure of these birds to aflatoxins, produced by fungi of the genus *Aspergillus* and a potential contaminant of peanuts (Lawson et al., 2006b). These authors tested liver samples from 13 greenfinches and 22 house sparrows that had been found dead at garden feeding stations, mostly dying from

salmonellosis or predation. Residues of aflatoxins (mostly aflatoxin B1) above detectable threshold levels were found in the liver of four house sparrows and seven greenfinches, but none of the livers had gross lesions suggestive of aflatoxicosis and histopathology was not possible because the livers had been frozen. The significance of these findings was therefore unclear.

3.2.13 Miscellaneous conditions and pathogens

Beak deformities

Different beak abnormalities encountered in house sparrows, great tits and blue tits were illustrated by Pomeroy (1962). These included crossing of the tips, elongations and curvatures. Genetic defects, trauma and infectious agents were considered to be possible causes.

Avian gastric yeasts (“megabacteria”)

Organisms described as “megabacteria” were observed on microscopy of wet preparations from the proventriculus of 15 greenfinches and a siskin found dead in Scotland (Pennycott* et al., 1998). “Megabacteria” are large Gram-positive filamentous organisms measuring 20-70 µm by 1-5 µm, found in the proventriculus of many species of bird, especially captive psittacines and passerines (Gerlach 2001). Their identity was unclear for many years, but they have now been classified as novel anamorphic ascomycetous yeasts and named *Macrorhabdus ornithogaster* (Tomaszewski et al., 2003). Although morphologically resembling a filamentous fungus, no fungal hyphae or ascospores were demonstrated, and phylogenetic analysis placed the organism within the ascomycetous yeast clade (Tomaszewski et al., 2003). These avian gastric yeasts (AGYs) can be associated with thickening and ulceration of the mucosa of the proventriculus, which becomes covered by a thick layer of mucus, and the integrity of the koilin layer of the gizzard becomes compromised (Gerlach 2001). They can, however, be incidental findings, as in the finches in Scotland, most of which had died from salmonellosis (Pennycott* et al., 1998). AGYs were also described in a wild purple

finch (*Carpodacus purpureus*) that died from trichomonosis in Canada (Forzan et al., 2010) but the significance was uncertain.

Encephalitis of unknown aetiology

An encephalitis affecting fledgling starlings, and to a lesser extent fledgling house sparrows, was described by Pennycott* et al. (2002b). Affected birds were invariably found in May or June and showed abnormalities of the central nervous system such as torticollis, ataxia or circling. Histopathological examination of the brain of one affected house sparrow showed a non-suppurative encephalitis suggestive of a viral or protozoal infection. Similar findings were made in six starlings, but no aetiological agent was identified.

Screening for Mycoplasma spp.

Small numbers of finches and sparrows were included in a batch of wild birds screened for mycoplasmas (Pennycott* et al., 2005b). No mycoplasmas were detected in the five sparrows or three finches tested, although *Mycoplasma sturni* was frequently isolated from blackbirds, starlings and corvids.

3.2.14 Screening UK small garden birds for potentially zoonotic bacteria

The potential threat to humans posed by wild birds infected with *C. psittaci* has been described above, and *Salmonella* Typhimurium is discussed in Chapter 4. Foster* et al. (2006) screened 231 samples of pooled faeces collected at a wild bird feeding station for verocytotoxin-producing *E. coli* (VTEC) O157, another bacterium that can adversely affect human health. Although only one sample yielded *E. coli* O157, the authors commented that wild birds could transfer the organism between livestock units or pass the bacterium to humans.

Hughes et al. (2009) screened wild bird faecal samples for the presence of *Campylobacter* spp., which are important causes of enteritis in humans. Over 2000 samples, including 154 from great tits, 86 from house sparrows, 84 from chaffinches and

93 from greenfinches, were collected from the north of England. *C. jejuni* was detected in a great tit, a house sparrow and a chaffinch, and *C. lari* in a greenfinch, giving an overall detection rate of slightly under 1%. This result was similar to that reported by Waldenstrom et al. (2002), who screened migrating birds in Sweden and isolated *C. jejuni* or *C. coli* from only 1.9% of 155 finches and sparrows screened. Using MLST analysis, Hughes et al. (2009) concluded that wild birds could provide a source of infection for livestock and humans, but that most spread was likely to be from livestock to wild birds.

Staphylococcus aureus is another important pathogen of humans and livestock, and the threat posed by antimicrobial-resistant strains, especially meticillin-resistant *S. aureus* (MRSA) is well documented. MRSA can be detected by standard microbiology supported by antimicrobial susceptibility testing, or by molecular techniques such as PCR to detect the *mecA* gene that confers resistance. In 2011, the demonstration of a strain of MRSA that was not detected using the routine PCR for the *mecA* gene was reported by Garcia-Alvarez et al. (2011). This novel MRSA with the gene *mecA* LGA251 was found in bulk milk from dairy farms in the UK and in humans from the UK and Denmark. Retrospective screening of isolates of *S. aureus* from other sources detected this new strain in a wide range of hosts, including rats, sheep, a common seal, a rabbit and a chaffinch from Scotland (Paterson* et al., 2012). The chaffinch isolate was an incidental finding in a bird that had died from trichomonosis but was the first report of MRSA in a wild bird (Robb* et al., 2013). Wild birds may therefore be one route by which MRSA could spread between livestock units or to humans.

3.3 Diseases of finches, sparrows, buntings, dunnocks and tits examined at Ayr DSC 1994 to 2013 – results

3.3.1 Bird species, numbers and locations

1278 finches, sparrows, buntings, dunnocks and tits were examined between January 1994 and December 2013. Of the 1243 birds for which a location was identified, 38.5% were found in the north of Scotland and 61.5% in the south. A summary of the species examined and the conditions diagnosed can be found in Tables 3.1 – 3.5. The materials and methods are described in Chapter 2 and the diagnostic criteria used are listed in Appendix II. “NOS” denotes conditions “not otherwise specified”. Images where indicated are in Appendix X.

Table 3.1: Finches, sparrows, buntings, dunnocks and tits examined.

Species	Number examined
Greenfinch	484
Chaffinch	312
Siskin	210
Goldfinch	53
Bullfinch	17
Lesser redpoll	11
Brambling	3
Crossbill sp.	1
House sparrow	110
Tree sparrow	7
Yellowhammer	3
Reed bunting	1
Dunnock	23
Blue tit	20
Coal tit	12
Great tit	9
Long-tailed tit	2
Total	1278

Table 3.2: Conditions* diagnosed in finches and buntings (n=1095).

Condition	Number of birds	% (95% confidence intervals)
Salmonellosis #	284	25.9 (23.4-28.7)
Salmonellosis (presumed) #	60	5.5 (4.2-7.0)
Trichomonosis (presumed or confirmed) #	373	34.1 (31.3-37.0)
<i>Escherichia albertii</i> bacteraemia #	151	13.8 (11.8-16.0)
Trauma	152	13.9 (11.9-16.1)
Miscellaneous conditions and pathogens:		All <2%
Adverse environmental conditions	13	
Cutaneous papilloma	11	
<i>Pasteurella multocida</i> infection	8	
Digestive tract condition not otherwise specified	5	
<i>Yersinia pseudotuberculosis</i> infection	3	
Chlamydiosis/chlamydiasis	3	
Reproductive tract disorder	3	
Cellulitis	3	
Feather/skin abnormality not otherwise specified	1	
Neoplasia	1	
Candidiasis	1	
No diagnosis	57	5.2 (4.0-6.7)
Incidental findings:		Not applicable
<i>Escherichia albertii</i> (incidental) #	23	
<i>Salmonella</i> Typhimurium through selenite only #	12	
Isolation of <i>Yersinia enterocolitica</i>	Not recorded	
Small to large numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Avian gastric yeasts in proventriculus of greenfinches	28	
Larval and nymphal ticks from chaffinches	5	
MRSA from chaffinch	1	
Oropharyngeal swabs from finches screened for mycoplasmas - no positive results	34	
Tissues screened for avian influenza virus – no positive results	335	
Tissues screened for West Nile virus – no positive results	216	

*Not all birds were examined for all conditions, and some birds had multiple conditions

see Chapters 4-7

Table 3.3: Conditions* diagnosed in sparrows (n=117).

Condition	Number of birds	% (95% confidence intervals)
Salmonellosis #	57	48.7 (39.4-58.1)
Trauma	25	21.4 (14.6-30.1)
Fledgling central nervous system disorder	8	6.8 (3.2-13.4)
Trichomonosis (presumed or confirmed) #	6	5.1 (2.1-11.3)
Pox (presumed or confirmed)	6	5.1 (2.1-11.3)
Coccidiosis	3	2.6 (0.7-7.9)
Miscellaneous conditions and pathogens:		All <2%
Cellulitis	2	
Central nervous system condition not otherwise specified	2	
<i>Pasteurella multocida</i> infection	1	
<i>Staphylococcus aureus</i> infection	1	
Significant helminthosis	1	
Feather/skin abnormality not otherwise specified	1	
Capture myopathy	1	
No diagnosis	10	8.5 (4.4-15.5)
Incidental findings:		Not applicable
<i>Salmonella</i> Typhimurium through selenite only #	2	
Isolation of <i>Yersinia enterocolitica</i>	Not recorded	
Small numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Schistosome-like eggs in intestinal contents	1	
Oropharyngeal swabs screened for mycoplasmas – no positive results	8	
Tissues screened for avian influenza virus – no positive results	32	
Tissues screened for West Nile virus – no positive results	35	

*Not all birds were examined for all conditions, and some birds had multiple conditions.

see Chapters 4-7

Table 3.4: Conditions* diagnosed in dunnocks (n=23).

Condition	Number of birds	% (95% confidence intervals)
Trauma	13	56.5 (34.9-76.1)
Salmonellosis #	4	17.4 (5.7-39.6)
Trichomonosis (presumed or confirmed) #	3	13.0 (3.4-34.7)
Pox	2	8.7 (1.5-29.5)
Miscellaneous conditions and pathogens:		All <5%
Significant helminthosis	1	
<i>Pasteurella multocida</i> infection	1	
Incidental findings:		Not applicable
Schistosome-like eggs in intestinal contents	2	
Hairworm eggs in intestinal contents	2	
Tissues screened for avian influenza virus – no positive results	8	
Tissues screened for West Nile virus – no positive results	6	

*Not all birds were examined for all conditions, and some birds had multiple conditions

see Chapters 4-7

Table 3.5: Conditions* diagnosed in tits (n=43).

Condition	Number of birds	% (95% confidence intervals)
Trauma	14	32.6 (19.5-48.7)
Digestive tract condition not otherwise specified	5	11.6 (4.4-25.9)
<i>Suttonella ornithocola</i> infection	4	9.3 (3.0-23.0)
Salmonellosis #	3	7.0 (1.8-20.1)
Adverse environmental conditions	3	7.0 (1.8-20.1)
Miscellaneous conditions and pathogens:		All <5%
Trichomonosis (presumed or confirmed) #	1	
Feather/skin abnormality not otherwise specified	1	
<i>Pasteurella multocida</i> infection	1	
Chlamydiosis/chlamydiasis – 1 pool of 4 birds	1	
No diagnosis	14	32.6 (19.5-48.7)
Incidental findings:		Not applicable
Tissues screened for avian influenza virus – no positive results	9	
Tissues screened for West Nile virus – no positive results	10	
<i>Salmonella</i> Typhimurium through selenite only #	1	
<i>Campylobacter jejuni</i> Penner Type 19	4	

*Not all birds were examined for all conditions, and some birds had multiple conditions

see Chapters 4-7

3.3.2 Trauma and pasteurellosis

Trauma – overview

Not unexpectedly, trauma was commonly diagnosed, being found in 152 finches, 25 sparrows, 13 dunnocks and 14 tits (16.0% [95% CI: 14.0-18.1] of submissions). Typical traumatic lesions included rupture of the heart or liver; blood in the chest, abdomen, lungs, mouth or trachea; puncture wounds and skin lacerations; and fractured bones.

Trauma in chaffinches

Traumatic lesions were found in 77 chaffinches, 24.7% (95% CI: 20.1-29.9) of all chaffinches submitted. The commonest primary causes identified were window collision (48 birds, based on history given at time of submission) and cat or raptor predation (13 birds, based on history or on the presence of punctures in the carcass). Evidence of a pre-existing infectious disease (salmonellosis, trichomonosis, pseudotuberculosis) was found in nine birds, five of which had been caught by predators. Peaks in February/March and in June were noted (Figure 3.1). Male (M) and female (F) adult chaffinches were equally represented (31M:28F) and most immature birds with trauma were submitted in June and July (Figure 3.2).

Figure 3.1: Trauma in chaffinches, by month.

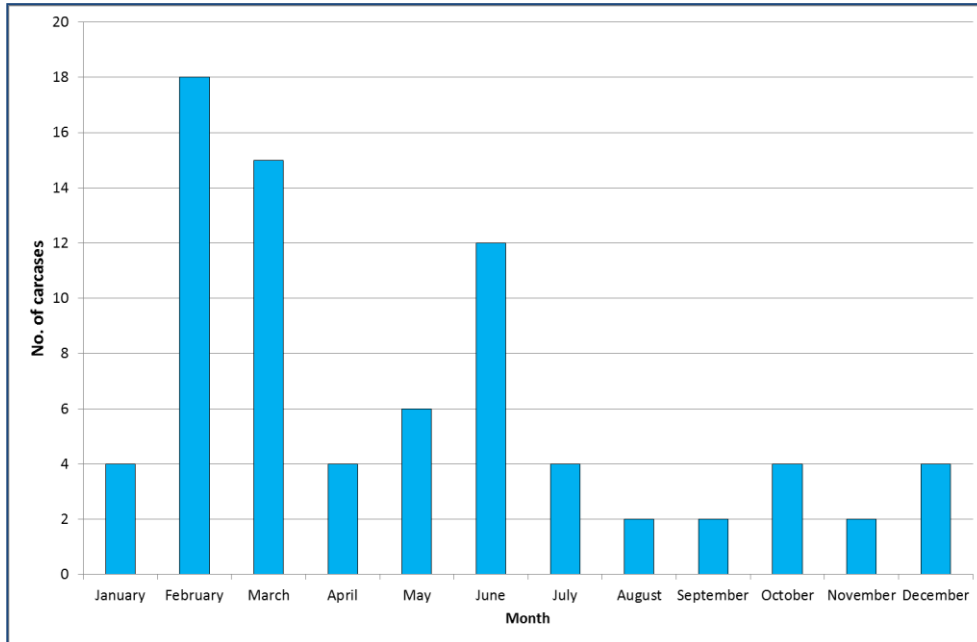
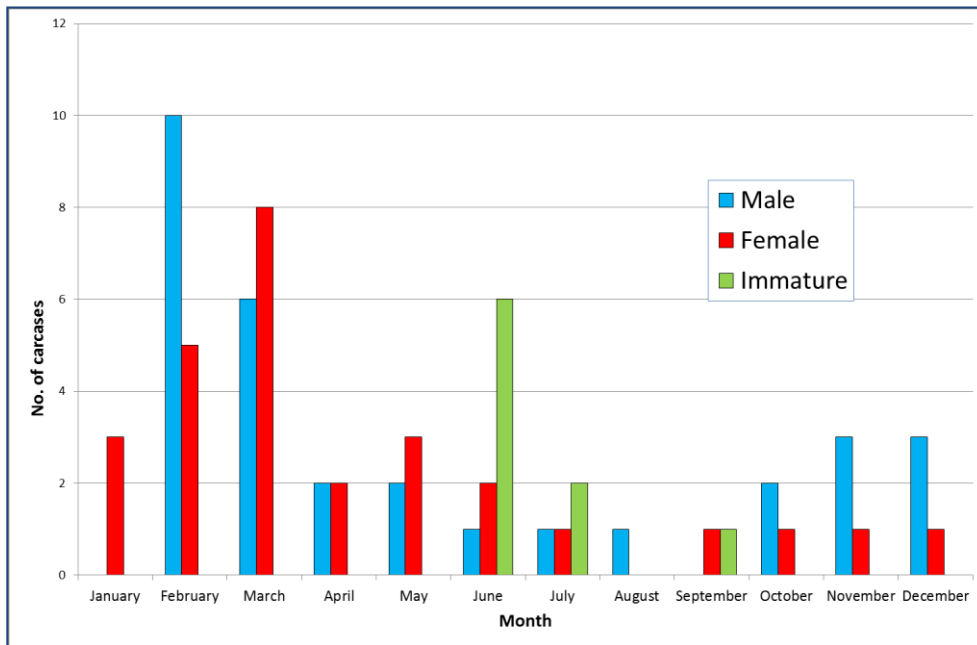


Figure 3.2: Trauma in chaffinches, by sex/age (where known) and month.



Trauma in greenfinches

Trauma was recorded in 35 greenfinches (7.2% [95% CI: 5.2-10.0] of greenfinch submissions). The cause was often not determined, but window collision (10 birds) and predator (4 birds) were the commonest causes. Only one bird had evidence of a pre-existing condition (salmonellosis). No clear seasonal pattern was apparent (Figure 3.3). Nearly three times as many male birds as females were affected (14M:5F); this was not statistically significant ($p=0.058$, two-tailed binomial test assuming males and females are equally represented in the population), but may have been significant had the sample size had been greater. Immature birds tended to be affected later in the season than chaffinches, most cases being seen in July to September (Figure 3.4).

Figure 3.3: Trauma in greenfinches, by month.

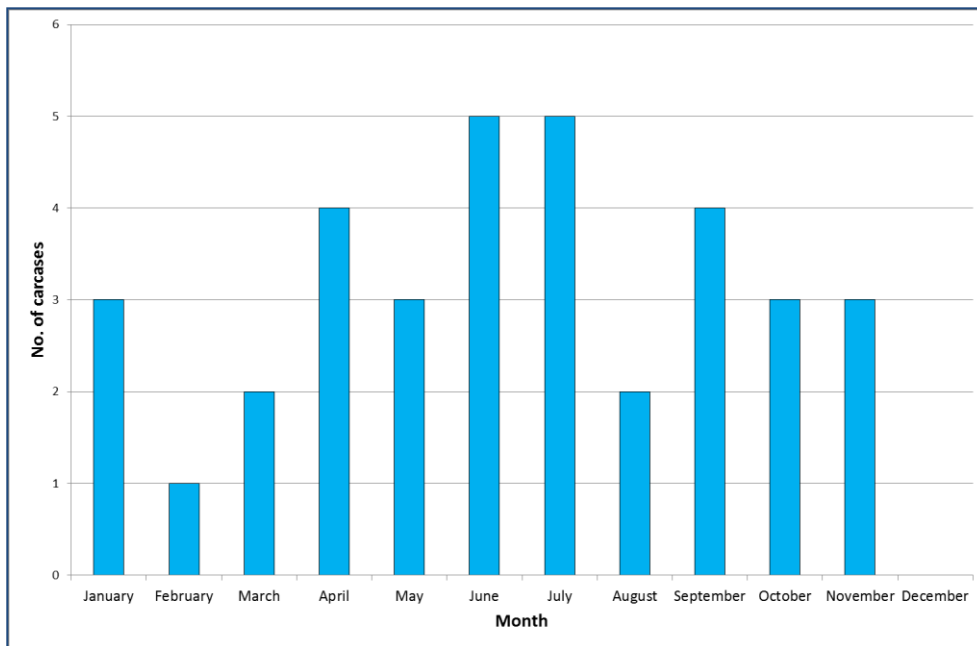
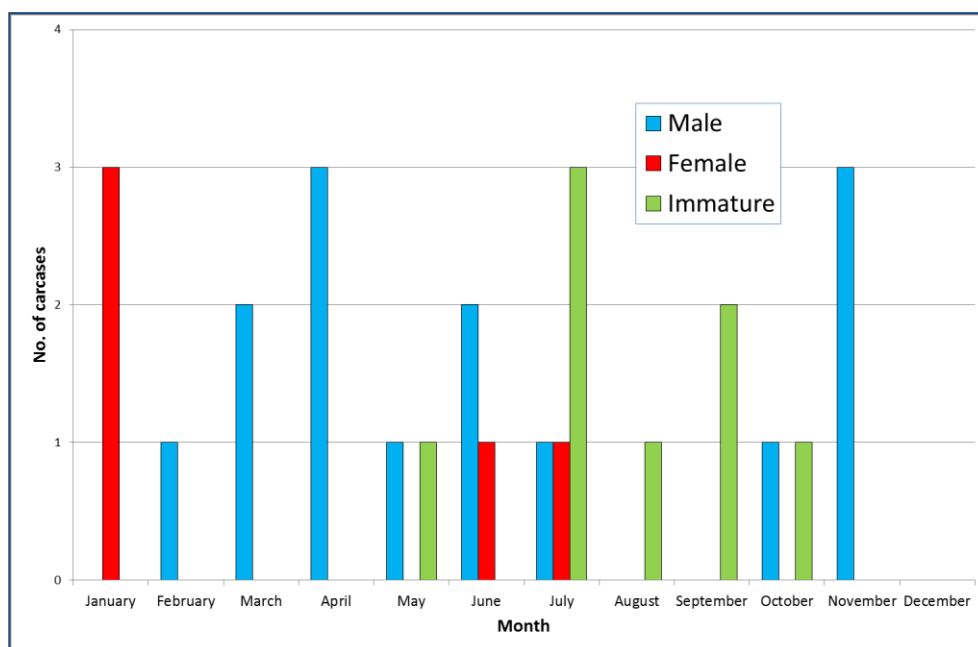


Figure 3.4: Trauma in greenfinches, by sex/age (where known) and month.



Trauma in siskins

Trauma was the cause of death of 22 siskins (10.5% [95% CI: 6.8-15.6] of siskin submissions). Most cases occurred in February to May, with a further peak in July (Figure 3.5). Males greatly outnumbered females (9M:1F, $p=0.016$) and peaks were seen in February/March (males) and July (immatures) (Figure 3.6). Where the cause was known, most (14 birds) had died after colliding with a window or patio door. Two had died from cat or raptor predation, and only one bird had a pre-existing infectious disease (salmonellosis).

Figure 3.5: Trauma in siskins, by month.

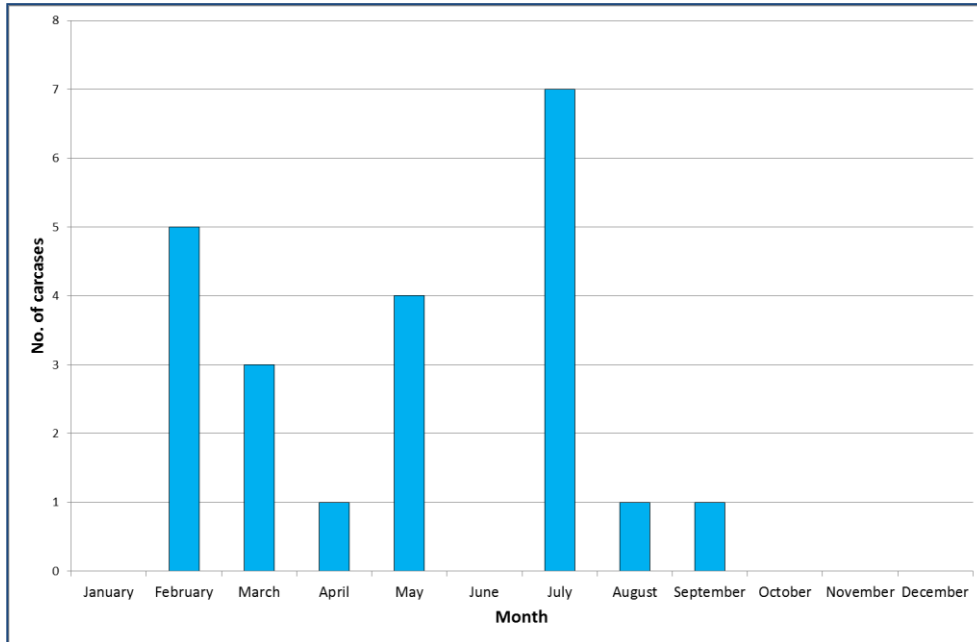
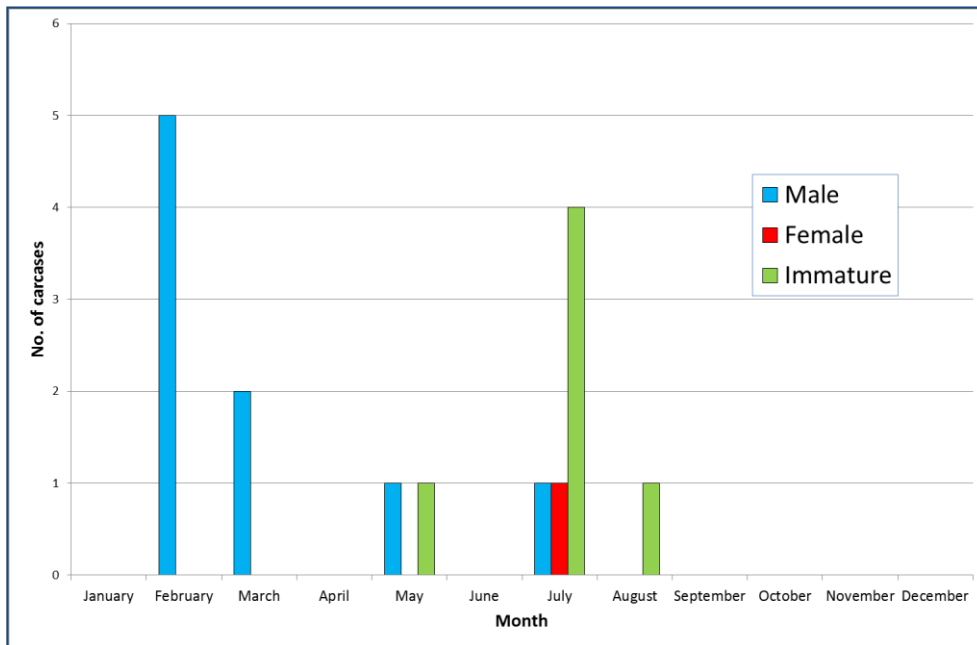


Figure 3.6: Trauma in siskins, by sex/age (where known) and month.



Trauma in house sparrows

Traumatic lesions were found in 23 house sparrows (20.9% [95% CI: 14.0-29.9] of house sparrow submissions), with no clear seasonal pattern (Figure 3.7). The sex was established in a small number of birds only, and males and females were equally represented. Trauma was recorded in immature birds in May to August (Figure 3.8). Primary causes were almost equally split between window collision (7 birds) and predation (6 birds) and lesions of a pre-existing infectious disease were only found in one bird (pox). One immature house sparrow was found alive with a head tilt and died shortly after. A puncture was present at the base of the neck accompanied by subcutaneous haemorrhage, suggestive of predator attack. In addition the breast muscle was flecked with small pale streaks approximately 1mm in length, and histopathology demonstrated multifocal areas of acute myodegeneration of the pectoral muscles. There was no evidence of parasitic involvement and no inflammatory reaction, and cardiac and gizzard muscle appeared normal. The lesions were ascribed to exertional or capture myopathy (Williams and Thorne, 1996).

Figure 3.7: Trauma in house sparrows, by month.

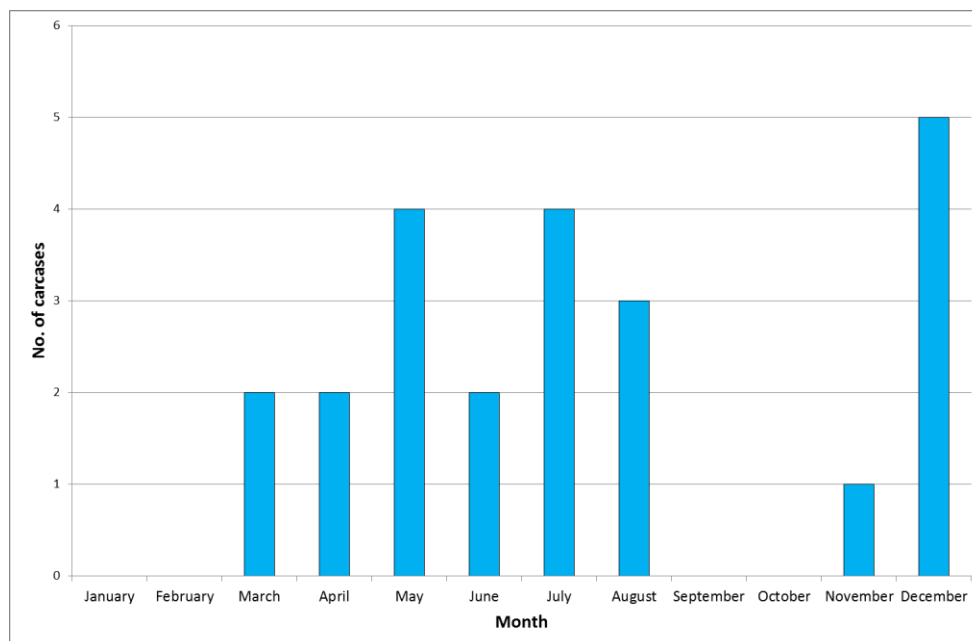
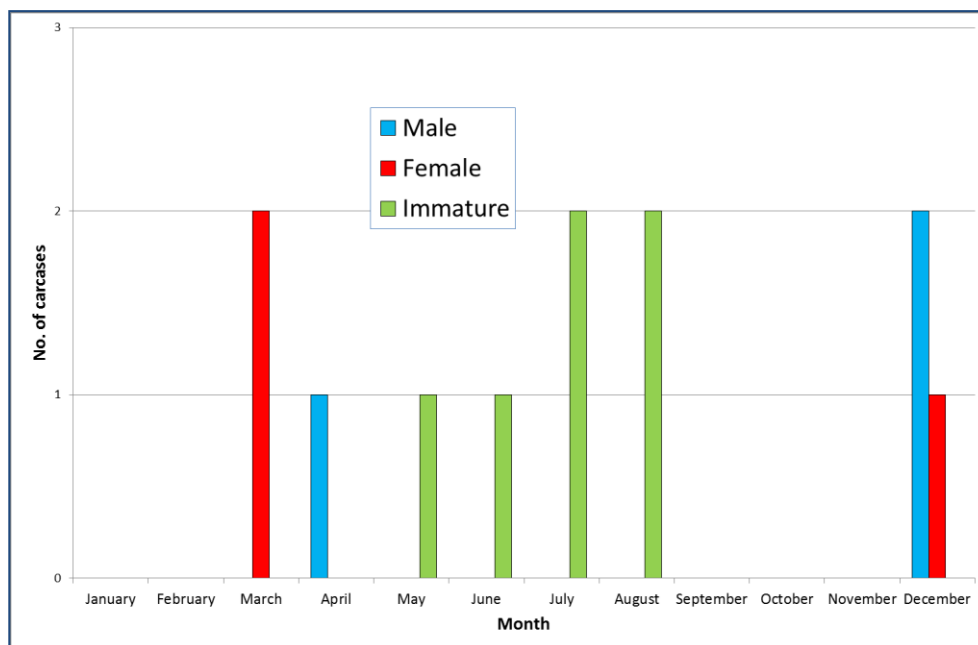


Figure 3.8: Trauma in house sparrows, by sex/age (where known) and month.



Trauma in other small garden birds

Trauma was also recorded in 8/53 goldfinches (15.1%), 7/17 bullfinches (41.2%), 13/23 dunnocks (56.5%), 14/43 tits (32.6%) and 2/7 tree sparrows (28.6%). Most of the recorded causes of the trauma were either predator attack or window collision, and in none of the birds was a significant predisposing infectious disease found.

Pasteurellosis

Pasteurella multocida was isolated from the liver, and sometimes also small intestine, of five greenfinches, a bullfinch, a siskin, a chaffinch, a dunnock, a house sparrow and a coal tit. In eight of the eleven birds, traumatic lesions were present and a history of cat predation was known or suspected, and cat predation could not be discounted in the remaining three birds.

3.3.3 Infection with *Salmonella* Typhimurium

See Chapters 4 and 7 for the review, results and discussion.

3.3.4 Infection with *Escherichia albertii*

See Chapters 5 and 7 for the review, results and discussion.

3.3.5 Trichomonosis

See Chapters 6 and 7 for the review, results and discussion.

3.3.6 Infection with *Suttonella ornithocola*

S. ornithocola was isolated from three out of 11 coal tits from which liver and intestine, and sometimes lung, were cultured, and from one out of 19 blue tits. The positive coal tits were submitted in the months January, February and April, and the blue tit in February. In two coal tits the organism was considered to be an incidental finding in birds that died during adverse weather conditions: small growths were recovered from the heart, lung, liver and intestine of one bird, and liver and intestine but not lung of the other bird. Histopathology of the lungs of these birds showed congestion but not pneumonia. *S. ornithocola* was isolated in heavy growth from the lung and liver of a third coal tit, in which histopathology suggested bacterial multiplication in these tissues after the death of the bird, and the significance was uncertain. In only one bird, a blue tit from which a heavy growth of *S. ornithocola* was recovered from the lung, was the presence of the organism associated with a multifocal necrotising pneumonia. The four birds were from different locations, but it is interesting to note that the blue tit and third coal tit were submitted approximately two months apart from two sites within 15 miles of each other. *S. ornithocola* may have been present in other tits submitted during the course of this study, but not recorded due to difficulties in isolating and identifying the organism; for example, two more coal tits submitted in April had heavily congested or consolidated lungs but *S. ornithocola* was not detected and no histopathology was carried out due to advanced autolysis.

3.3.7 Infection with *Chlamydia (Chlamydophila) psittaci*

Eight finches from five locations were screened by PCR for the presence of *C. psittaci*. Chlamydiosis had been considered in one or more finches on those sites on the basis of postmortem lesions (airsacculitis, liver necrosis or green fluid intestinal contents) or the presence of confirmed chlamydiosis in robins on the site. The organism was detected in two greenfinches and a chaffinch. All three birds (from three different locations) had diffuse thickening of the oesophagus typical of trichomonosis. The presence of *C. psittaci* was considered to be significant in the chaffinch, which had a fibrino-necrotic airsacculitis, and in one greenfinch with green watery intestinal contents, but was probably incidental in the other greenfinch. *C. psittaci* was also demonstrated by PCR in pooled tissues from four blue tit nestlings found dead in their nest – large fragments of peanuts were present in the gizzard and small intestine of all the birds, likely to be the cause of enteritis and death, and the demonstration of *C. psittaci* was believed to be an incidental finding.

3.3.8 Avian pox, cutaneous papillomas and other skin conditions

Lesions typical of avian pox were found in two dunnocks and six house sparrows. One dunnock had multiple nodules on both feet (**Image 1**), and the second dunnock had a large mass below one eye and smaller nodules in the skin of the neck (**Images 2-4**). Wart-like nodules were typically found around the eyes, the crown of the head and at the commissures of the beak of the house sparrows (**Images 6-7**). Less commonly the skin of the neck, under the wings and of the thighs were affected. Histopathology (**Image 5**) confirmed the diagnosis in three birds from which samples were taken. No seasonal pattern was apparent for birds with pox.

Eleven chaffinches, some of which had died from salmonellosis or trauma, had lesions suggestive of cutaneous papillomas affecting one or both lower legs (**Images 8-12**). Verrucose masses surrounded the digits, sometimes with only the claws protruding. Small nodules were occasionally found on the legs. Histopathology was carried out on lesions from three birds, confirming the diagnosis. Cnemidocoptic mites were not

detected on histopathology or on KOH preparations and further examination by electron microscopy demonstrated papilloma-like viruses in one bird. All eleven affected birds were submitted between January and May, suggesting a seasonal pattern.

Three birds had feather/skin conditions of uncertain causes. A chaffinch had feather loss affecting the head, neck and upper wings, but KOH preparations did not detect any mites or ringworm spores and no significant bacteria or fungi were isolated. Feather loss and scabbing of the head and neck was seen in a blue tit. Large yellow/white adherent crusts were present on one side of the head and around eyes, and although fluorescence under UV light was apparent, cultures and microscopy for ringworm were negative. No significant bacteria, fungi or ectoparasites were found. The third bird was a house sparrow which had thickening of the skin of the head and extensive orange thickening and fissuring of the skin of one thigh ([Images 13-14](#)). Examination for ectoparasites and ringworm was unrewarding, but mixed bacteria including heavy growths of *Staphylococcus aureus* were recovered from the liver and skin. Concurrent lesions of trichomonosis were also present.

3.3.9 Helminths, coccidia and avian gastric yeasts (“megabacteria”)

No helminths were observed grossly in any of the finches, dunnocks or tits examined in this study, but four pairs of large syngamid worms presumed to be *Syngamus trachea* were found obstructing the trachea of an adult house sparrow in July ([Images 15a-16b](#)). The bird had been observed to be coughing prior to death. A few hairworms or hairworm eggs ([Images 38a-c](#)) were detected in the intestinal contents of two dunnocks: one had died from salmonellosis, the other from trauma. Large numbers of structures provisionally identified as schistosome-like eggs ([Images 22-32](#)) were present in the intestinal contents of a thin adult dunnock with very fluid intestinal contents and a soiled vent, and were considered to be significant. Similar eggs were detected in the mucosa and intestinal contents of two thin dunnocks that had died from trichomonosis, and in the intestinal contents of a thin house sparrow that had died from salmonellosis ([Images 33-36](#)). The eggs were spherical to sub-spherical or ellipsoid, with a thin smooth outer

membrane. The contents were dark and granular, either filling the egg or sometimes retracted to produce a spherical or ellipsoid mass with a clear space between the contents and the outer membrane. They were variable in size, with the widest dimension ranging between 59 μm and 105 μm (three dunnocks) and 61 μm and 110 μm (one house sparrow). No helminths were observed grossly in these birds and autolysis unfortunately precluded histopathology. Similar eggs were also found in blackbirds and a redwing (*Turdus iliacus*), and the rationale for provisionally identifying them as schistosome-like eggs is further discussed in Chapter 8.

Wet preparations were made from the small intestine of 120 finches and examined for coccidial oocysts, helminths and helminth eggs. No helminths or eggs were seen, but small to large numbers of oocysts of isosporoid coccidia (Images 17-20) were found in many birds. In all cases they were considered to be incidental findings in birds dying from a range of other conditions such as salmonellosis, *E. albertii* bacteraemia and trauma. Coccidial oocysts were also found in intestinal smears from several house sparrows, including large numbers in three thin birds (one immature and two adults in September to November) with fluid pink or yellow intestinal contents. In the absence of other pathological findings, the presence of large numbers of coccidial oocysts in these birds was considered to be significant. Further details about the helminths, eggs and coccidia detected can be found in Appendix III.

Proventricular smears were made from 86 finches, six tits and two house sparrows. Organisms with the morphology of “megabacteria” (avian gastric yeasts, AGYs) (Image 21) were demonstrated in 29 greenfinches, two siskins and one goldfinch (37.2% [95% CI: 27.2-48.3] of finches screened), but not in any tits or house sparrows. The greenfinches came from eight different sites and in most cases the AGY were believed to be incidental findings in birds dying from salmonellosis or trauma, and the siskins and goldfinch with AGY were from two sites and had died from *E. albertii* bacteraemia. However, in four immature greenfinches submitted from one site and within a few days of each other, the presence of large numbers of AGY was associated with excess thick

mucus in the proventriculus. Two of these birds had thickening and distortion of the proventriculus, and in one bird the koilin layer of the gizzard was thickened and ulcerated. Three of the four birds had concurrent salmonellosis. Histopathology of the proventriculus of one affected bird showed a mixed lymphoid and granulocytic infiltration and many AGYs on the mucosal surface and within the proventricular glands, but it was unclear if the inflammatory reaction was caused by the salmonellosis or the AGYs. In these four birds the AGYs were believed to be contributing to the gross pathology observed. A fifth immature bird from this cohort was also thin and had large numbers of AGYs, but in the absence of gross pathology and failure to demonstrate *S. Typhimurium* this was recorded as “no diagnosis”.

3.3.10 Disorders of the central nervous system

Ten fledgling house sparrows were received with the history of central nervous system (CNS) disorders. All were submitted in the months May to July, and exhibited signs such as torticollis, circling and ataxia. Histopathology typically showed a non-suppurative encephalitis, with perivascular cuffing by predominantly mononuclear inflammatory cells, gliosis, vasculitis, thrombosis and areas of malacia or necrosis of the neuropil. The mononuclear inflammatory response was suggestive of a viral or protozoal aetiology and in one bird round structures suggestive of protozoa were associated with the perivascular cuffs. A diagnosis of “Fledgling CNS disorder” was recorded in these birds. In two other birds the condition was recorded as “CNS condition not otherwise specified” because heavy granulocytic infiltration was also present, but these were possibly later stages of the same condition. This disorder mostly affects starlings but house sparrows are also susceptible, and the aetiology has yet to be determined (Pennycott* et al., 2002b).

3.3.11 Adverse environmental conditions

In 13 finches (8 chaffinches, 3 greenfinches, 1 bullfinch, 1 siskin), two coal tits and a blue tit, death was attributed to adverse environmental conditions. Usually these were thin or wet birds found during periods of prolonged cold weather, torrential rain etc., but

also included birds inadvertently trapped inside buildings. *S. ornithocola* was isolated from the two coal tits but was not considered to be significant.

3.3.12 Miscellaneous conditions and pathogens

Digestive tract conditions not otherwise specified (NOS)

Digestive tract conditions NOS included pathology associated with avian gastric yeasts (see above). The cause of yellow caseous thickening of the hard palate and beneath the tongue of an adult blue tit was not determined. Wet preparations did not detect any protozoa and bacteriology yielded *Chryseomonas luteola* of doubtful significance. Histopathology showed stomatitis associated with many bacteria but no protozoa or other organisms. “Digestive tract condition NOS” was also diagnosed in a brood of four blue tit nestlings with fluid intestinal contents, most likely caused by large fragments of peanuts in the gizzards and intestines, and in an adult yellowhammer that died after a large pea obstructed the upper oesophagus.

Reproductive tract disorders

Reproductive tract disorders were noted in three birds. A goldfinch and a chaffinch had egg peritonitis, with active reproductive tracts and smearing of the abdominal cavity with yolk material. A greenfinch had an impacted oviduct in addition to egg peritonitis.

Neoplasia

A thin adult chaffinch had an enlarged liver with multiple pale miliary foci, and a greatly enlarged pale spleen. A bacterial condition was initially suspected but no significant organisms were isolated and a PCR for *C. psittaci* was negative. Histopathology, however, demonstrated changes consistent with lymphosarcoma in liver and spleen.

Yersinia pseudotuberculosis

A thin chaffinch had a large caseous mass around one shoulder joint - *Y. pseudotuberculosis* was isolated from the liver and mass. A heavy growth of the same organism was recovered from the liver of a thin chaffinch that died after being predated.

In both birds the presence of the organism was likely to be significant. Mixed growths of *Y. pseudotuberculosis* and *S. Typhimurium* were demonstrated in the liver and intestine of a third chaffinch, but on that occasion the latter organism was considered the more significant. The three cases occurred in January to March.

Cellulitis

In addition to the cellulitis and/or arthritis in some birds caused by *T. gallinae*, *Y. pseudotuberculosis* and *S. Typhimurium*, cellulitis was noted in two other finches; a greenfinch had cellulitis under the skin over the abdomen, and caseous cellulitis was found at the base of the neck of a siskin. Heavy growths of *E. coli* were isolated from the liver, intestine and infected tissues of both birds, but the underlying cause was not determined.

Candidiasis

A chaffinch with trichomonosis affecting the oesophagus also had a large caseous mass at the side of the face, extending to the maxilla, hard palate and oropharynx. Mixed bacteria and *Candida albicans* were isolated (Images 94-96).

3.3.13 No diagnosis

No diagnosis was made in 57 finches, 10 sparrows and 14 tits (6.3%). In some finches and sparrows, salmonellosis was suspected, but *S. Typhimurium* was not isolated or only recovered through enrichment and so the diagnostic criteria were not met. Similarly there were other finches with oesophageal lesions suggestive of salmonellosis or trichomonosis but no cultures were made, not fulfilling the diagnostic criteria of either condition. *E. albertii* bacteraemia was suspected in several siskins in which the cultures were overgrown by other non-lactose fermenting bacteria. Some “no diagnosis” cases were “orphaned” or “abandoned” nestlings or fledglings that died after being “rescued” by the general public. Several “no diagnosis” cases were birds found dead in good condition and with food in the digestive tract and in which trauma was suspected, and

others were thin, with no food, suggesting previous adverse environmental conditions or earlier trauma had interfered with the birds' ability to feed.

3.3.14 Incidental findings

Incidental findings such as the non-significant presence of isosporoid coccidia and avian gastric yeasts in some birds have already been described, and incidental isolations of *S. Typhimurium* and *E. albertii* will be discussed in Chapters 4 and 5 respectively. Meticillin-resistant *Staphylococcus aureus* (MRSA) was demonstrated in a chaffinch with trichomonosis, and *Campylobacter jejuni* Penner Type 19 was recovered from the intestinal contents of four blue tit nestlings with enteritis associated with peanut fragments.

Small ticks were found on five chaffinches or in the packaging in which the birds were submitted. The birds were all thin and three had lesions consistent with trichomonosis. The ticks from three of the birds were identified as larval and nymphal stages of *Ixodes ricinus*, but were thought to be incidental findings. Two of the birds were submitted in March and May, the remaining three birds in September.

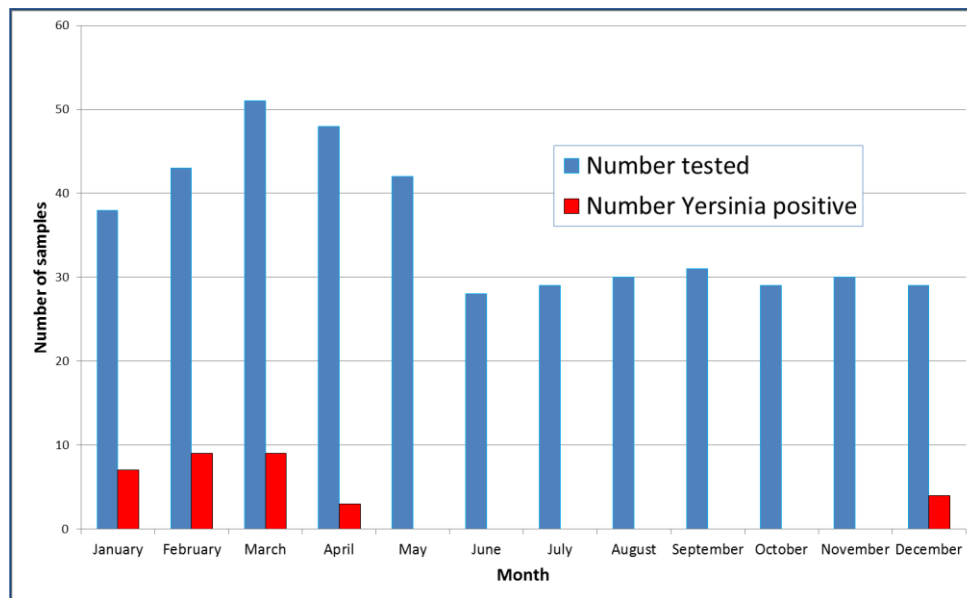
Between April 1999 and March 2003, oropharyngeal swabs from 34 finches and eight house sparrows were screened for mycoplasmas, as part of a study examining the potential role of wild birds as sources of mycoplasmas for poultry and gamebirds (Pennycott* et al., 2005b), but no mycoplasmas were detected.

Tissues from 384 birds were sent to AHVLA Weybridge to be screened for avian influenza viruses, but no positive results were reported. Similarly, tissues from 267 birds were sent to AHVLA to be screened for West Nile virus, but no positive results were received.

Yersinia enterocolitica was recovered as an incidental finding from the small intestine and/or liver of several finches and house sparrows. In addition, between October 2000

and September 2003, 428 samples of pooled faeces were collected from bird tables in Scotland and screened for bacteria as described by Pennycott* et al. (2002a). *Y. enterocolitica* was isolated from 32 samples, all in the months December to April (Figure 3.9). Four representative isolates were serotyped by the Public Health Laboratory Service (PHLS) Colindale as biotype 1a. Antimicrobial susceptibility test results for isolates from carcasses and faeces are shown in Appendix IV; resistance to some members of the beta lactam group of antimicrobials was not uncommon, especially in isolates from carcasses. Small numbers of isolates were also resistant to sulphisoxazole, but no isolate was resistant to more than one antimicrobial group.

Figure 3.9: Isolation of *Yersinia enterocolitica* from pooled faeces from bird tables



3.4 Discussion

3.4.1 General comments

Carcases were received from many different parts of Scotland, largely because of the ease of posting small carcasses. Although the greatest number (61.5%) originated from the south, the results may be representative of birds in other parts of Scotland. The results do not give an indication of the prevalence of different diseases in the population, because no denominator data are available and there is likely to be bias in carcass selection and submission. However, the sample size was sufficiently large that comparison between some different diseases in Scotland was possible (see Chapters 4-7). The literature review and results provide information to wildlife rehabilitators and their veterinary advisers about the conditions most likely to be encountered when dealing with birds of this category.

Salmonellosis, *E. albertii* bacteraemia and trichomonosis are discussed in Chapters 4-7, and some of the other conditions encountered such as pox, cutaneous papillomas and pasteurellosis need no further elaboration. Helminths were seldom found in finches, sparrows or tits, compared with starlings, blackbirds, thrushes and corvids (see Chapters 8 and 9): the latter groups rely more heavily on soil invertebrates including leatherjackets and earthworms that can act as intermediate or transport hosts for some helminths. Awareness of this distinction will aid wildlife rehabilitators and their veterinary advisers when formulating treatment and control programmes in such birds. Hairworms and schistosome-like eggs were, however, noted in five dunnocks, and in one dunnock the schistosome-like eggs were believed to be the cause of fluid intestinal contents and death. Similar eggs were also found in a house sparrow, six blackbirds and a redwing, and their identity and significance is discussed more fully in Chapter 8.

A similar split between the two groups of garden birds was noted for *Mycoplasma sturni*, isolated from blackbirds, starlings and corvids (see Chapters 8 and 9) but not from dunnocks, finches, sparrows or tits. Conversely, deaths from salmonellosis, *E.*

albertii bacteraemia and trichomonosis were almost all diagnosed in the latter group and very seldom found in the former (see Chapters 4-7). Fledgling CNS disorder of house sparrows is discussed more fully when considering the condition in starlings (Chapter 8). The potential hazard of birds eating unsuitable food from garden feeders was illustrated by the death of an adult yellowhammer in which the upper oesophagus was blocked by a large dried pea (possibly from a pigeon mixture?), and enteritis in a brood of blue tit nestlings was associated with large fragments of peanuts, presumably fed by the parent birds. These cases reinforce the message that care must be taken when selecting the types of supplementary feed offered at garden feeders, especially when adult birds are feeding their young or when more unusual birds are visiting the feeders. Some of the “negative” findings were also of significance, such as the lack of evidence of avian pox in great tits in Scotland, despite its presence in England, and the failure to detect avian influenza viruses or West Nile virus.

3.4.2 Potentially zoonotic organisms and antimicrobial susceptibility of bacteria recovered from finches, sparrows, buntings, dunnocks and tits

Several potentially zoonotic organisms were demonstrated in small garden birds during the surveillance period, including *S. Typhimurium*, *C. psittaci*, *E. coli* O157, *Campylobacter* spp., *Y. pseudotuberculosis* and *Y. enterocolitica*. The potential spread of *S. Typhimurium* from garden birds to humans, especially young children, was highlighted by Philbey et al. (2008) and Lawson et al. (2014), and is discussed more fully in Chapter 4. Garden birds may be a greater source of *C. psittaci* for humans than previously thought, and Foster* et al. (2006) reported that contact with faeces from garden birds could be a source of *E. coli* O157 for humans. The results of Hughes et al. (2009) suggested that garden birds were unlikely to be a major hazard as regards food poisoning caused by *Campylobacter* spp., but the demonstration of *Y. pseudotuberculosis* from three chaffinches in the current study is a reminder that wild birds could be a direct or indirect source of infection for humans, although there is no evidence to indicate that this is a common occurrence.

Y. enterocolitica can cause diarrhoea in humans, sometimes progressing to more serious complications such as mesenteric lymphadenitis, arthropathies, and (rarely) septicaemia and death (McNally et al., 2004). Consumption of meat, especially pork, and dairy products are the main sources of infection for humans, and McNally et al. (2004) found that nearly 30% of pigs slaughtered in GB were carrying *Y. enterocolitica*, as were smaller numbers of cattle and sheep. Different biotypes of *Y. enterocolitica* have been identified, and the commonest type recovered by McNally et al. (2004) was biotype 1a. This is also the commonest biotype recovered from humans with or without clinical signs of disease and this biotype has previously been thought to be non-pathogenic in humans and livestock. However, more recent studies cited by McNally et al. (2004) suggest that some strains of biotype 1a are capable of causing disease in humans. In the current study, *Y. enterocolitica* was recovered from 32 out of 428 samples of pooled faeces collected from bird tables, and this recovery rate is likely to be an underestimate of the true prevalence because no selective media or incubation temperatures were used to increase the likelihood of detection. It is interesting to note that all isolations were made in December to April, and McNally et al. (2004) found that faecal carriage in pigs was highest in December and January. Four isolates from wild bird faeces were subsequently identified by PHLS as biotype 1a, and given the current uncertainty of the role of this biotype in human disease, further molecular typing of human and wild bird isolates should be carried out. In a study in Germany looking at risk factors for sporadic cases of *Y. enterocolitica* gastro-enteritis in humans (Rosner et al., 2012), the main risk factor was the consumption of raw minced pork, but playing in a sandbox and recent contact with birds were also found to be significant risk factors in young children. Given the risks of infection of young children with *S. Typhimurium* and *Y. enterocolitica*, further work should also be carried out to determine if the isolates of *E. albertii* from finches in the UK (see Chapter 5) are pathogenic to humans, as it is worth remembering that this organism was first described in children in Bangladesh with diarrhoea (Huys et al., 2003).

A summary of the results of antimicrobial susceptibility tests for non-lactose-fermenting (NLF) bacteria is presented in Appendix IV. Some resistance to sulphonamides and tetracyclines was found in isolates of *S. Typhimurium* from carcasses and faeces (Tables 4-5), and small numbers of isolates of *E. albertii* were resistant to different groups of antimicrobials (Table 6). Resistance to some of the β lactam group of antimicrobials was common in isolates of *Y. enterocolitica*, and a few isolates were resistant to sulphonamides. The production of β lactamases by human and environmental isolates of *Y. enterocolitica* is well recognized and varies between biotypes of *Y. enterocolitica* (Pham et al., 2000), which may explain why the isolates from carcasses tended to show more β lactam resistance than isolates from the environment (Tables 10-12 in Appendix IV). Antimicrobial susceptibility results for other NLF organisms recovered from finches and sparrows are also summarised in Appendix IV (Tables 7-9). Overall, although evidence of resistance was found, no isolate was resistant to more than two different antimicrobial groups, and garden birds do not appear to be a major source of NLF bacteria carrying multiple resistance genes that could be spread to humans. Nevertheless, Robb* et al. (2013) described the first recovery of MRSA from a wild bird, a chaffinch in Scotland, highlighting that a small proportion of garden birds could harbour resistant organisms or genes.

In summary, the results of this 20 year study emphasised the importance of personal hygiene when cleaning garden bird feeding stations or handling the carcasses of garden birds, and that young children should be encouraged to wash their hands after playing near bird feeders.

3.4.3 Infection with avian gastric yeasts and *Suttonella ornithocola*

When this study commenced in 1994, the identity of “megabacteria” was unknown and the bacterium *S. ornithocola* had not been characterised or linked to deaths in tits. “Megabacteria” have now been classified as avian gastric yeasts (AGYs) and named as *Macrorhabdus ornithogaster*, and have been demonstrated in a wide range of avian species including psittacines, passerines, ostriches, chickens and Japanese quail

(Pennycott et al., 2003). In many cases their presence is believed to be incidental, but in some situations overgrowth of the organism can result in a proventriculitis. In the current study, AGYs were frequently demonstrated in greenfinches dying from salmonellosis or trauma and considered to be incidental. However, in four immature greenfinches submitted from a densely populated feeding station with an ongoing problem of salmonellosis, large numbers of AGYs were associated with proventricular abnormalities such as excess mucus production, thickening and distortion of the mucosa of the proventriculus, and thickening and ulceration of the koilin layer of the gizzard. Similar lesions were not seen in birds with salmonellosis from other sites, and the AGYs were believed to be playing a role in the pathology seen. Similar to the situation described by Pennycott et al. (2003) in chickens and Japanese quail, it appears that AGYs can be associated with pathology of the proventriculus and gizzard of finches that have concurrent infectious diseases and that have also been exposed to large numbers of AGYs.

S. ornithocola was isolated from three coal tits and a blue tit during the course of this study, but owing to the fastidious nature of the organism it may have been present but undetected in other tits. Kirkwood et al. (2006) noted that tit mortality incidents occurred from March to May, and Lawson* et al. (2011b) reported that mortality incidents associated with *S. ornithocola* occurred from January to April. In the current study *Suttonella*-positive birds were also submitted in January to April, adding weight to the suggestion of a seasonal pattern of mortality. It should be noted that lung histopathology of the four positive birds in this study demonstrated multifocal necrotising pneumonia in only one bird, a blue tit, and pneumonia was not found in the three coal tits, two of which had died during adverse weather conditions. Similarly, in the mortality incidents described by Lawson* et al. (2011b), pneumonia was restricted to three blue tits. It is possible, therefore, that coal tits carry this organism without showing clinical disease, and that blue tits are more likely to develop pneumonia. To explore this further, bacteriology and lung histopathology should be carried out on a larger number of samples from tits and other groups of birds.

3.4.4 Trauma

Trauma was commonly diagnosed in small garden birds, accounting for over 20% of diagnosable submissions of chaffinches, bullfinches, house sparrows, tree sparrows, tits and dunnocks. Fewer cases were diagnosed in greenfinches, siskins and goldfinches. The commonest causes identified were window collisions and predation by cats or raptors. Pre-existing infectious diseases (salmonellosis, trichomonosis, pseudotuberculosis, pox) were only found in 4.0% of cases of trauma, mostly in chaffinches. Where the sex was recorded in adult birds, equal numbers of male and female house sparrows and chaffinches were involved, but males outnumbered females by at least a factor of 2 in greenfinches, goldfinches and siskins. Male deaths from trauma were especially highest in February (siskins), March (goldfinches) and April (greenfinches), suggesting behavioural changes in males in the spring of the year may increase their susceptibility to trauma. When discussing the reasons for the sex ratio of birds dying from some infectious diseases, it is sometimes proposed that birds dying from trauma be used as a “control” population, assuming that males and females are equally likely to die from trauma (e.g. Lawson et al., 2010). However, the results of the current study show that for some species there can be seasonal variation in the sex ratio of birds dying from trauma, and this is discussed more fully in Chapter 7.

Chapter 4

Salmonellosis in UK finches, sparrows, buntings, dunnocks and tits

4.1 Introduction

Chapter 3 discussed some of the diseases encountered in UK finches, sparrows, buntings, dunnocks and tits. This chapter examines salmonellosis in this group of birds, and Chapters 5 and 6 cover *Escherichia albertii* bacteraemia and trichomonosis respectively. Further discussion about these three infectious conditions and their interaction is presented in Chapter 7. The Latin names of the UK birds discussed are listed in Appendix I.

4.2 Review of salmonellosis in UK finches, sparrows, buntings, dunnocks and tits

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

4.2.1 Early reports of salmonellosis in garden birds

Salmonellosis in UK garden birds in the 1960s was reported by Wilson and Macdonald (1967), Goodchild and Tucker (1968), and Macdonald and Cornelius (1969). Outbreaks affecting multiple birds at garden feeding stations were reported from different parts of England and Scotland, most commonly affecting house sparrows and greenfinches. Postmortem findings included oesophageal ulceration and necrosis; enlargement of liver and spleen; focal necrosis or abscessation in organs such as the liver, spleen and caecal tonsils; pericarditis; and panophthalmitis. Bacteriology demonstrated heavy growths of *S. Typhimurium*. Disease was thought to be the result of supplementary feeding attracting unusually large numbers of birds and causing increased bacterial contamination of the feeding stations.

4.2.2 Further incidents, changing phage types

Retrospective examination of records at Lasswade Veterinary Laboratory in Scotland, using the bacteriophage typing nomenclature published by Anderson et al. (1978),

showed that most isolates of *S. Typhimurium* from garden birds in the 1960s/1970s were definitive phage types (DTs) 40, 160, 129 and 37 (Pennycott* et al., 2010). Deaths became uncommon after the winter of 1975-1976 (Macdonald and Bell, 1980) but Laing (1990) reported multiple deaths from salmonellosis in house sparrows in an industrial plant, and in the winter of 1994/1995 there seemed to be a resurgence of salmonellosis in garden birds at feeding stations in different parts of England (Kirkwood et al., 1995; Routh and Sleeman 1995). In 1997 salmonellosis was diagnosed in 50 birds (49 greenfinches and a chaffinch) from seven gardens in the Grampian region of Scotland (Pennycott* et al., 1998). The postmortem lesions were as described in earlier outbreaks, and *S. Typhimurium* DT40 was consistently recovered from the carcasses.

During the monitoring of wild bird carcasses and faeces at two sites in southwest Scotland in 2000 and 2001, a different phage type of *S. Typhimurium*, designated DT56 variant (DT56v), was found to be endemic at one of the sites (Pennycott* et al., 2002a). This organism was recovered from 48% of pooled faeces collected from a bird table, 42% of faeces from under a hanging feeder, 33% of faeces collected from beneath a roost used by house sparrows, and from the carcasses of three chaffinches, two house sparrows and a tawny owl (*Strix aluco*). Four of the birds (two chaffinches and two house sparrows) had lesions typical of salmonellosis, and this was the first published report of mortality in garden birds caused by this phage type. Further monitoring at this site (Pennycott* et al., 2005a) showed that *S. Typhimurium* can persist at some sites for nearly three years, and that there was a significant positive correlation between the level of contamination of the feeding station and the number of house sparrows (but not greenfinches, chaffinches or blackbirds).

Salmonellosis in garden birds continued to be a problem, and Pennycott* et al. (2010) presented details of 198 incidents of salmonellosis in Scotland between September 1995 and August 2008. One hundred and eleven incidents were caused by DT40, 85 incidents by DT56v and two by DT41. There was a marked and statistically significant geographic split, with most (88%) incidents in the north arising from infection with DT40,

compared with 31% DT40 and 68% DT56v in the south of Scotland. Salmonellosis was seen in finches in both the north and south of Scotland, but disease in house sparrows was more commonly seen in the south of the country. New incidents of salmonellosis in chaffinches, regardless of phage type or region, occurred in January or February. New incidents of DT40 infection in greenfinches in both regions tended to occur in January to March, peaking in January/February. New incidents of DT56v in greenfinches tended to be more spread out, still peaking in January/February but occurring from November to March. Most new incidents of DT56v in house sparrows also occurred between November and March, but with no marked peaks. This analysis highlighted differences in the epidemiology of salmonellosis in garden birds, depending on species of bird, phage type of *S. Typhimurium*, and region of Scotland.

A retrospective analysis of salmonellosis incidents in England and Wales between 1993 and 2003 also showed regional differences in phage types (Lawson et al., 2010). Of those isolates that were phage typed, 54% were DT40, 29% were DT56v and 7% DT160. The incidents of DT40 and DT56v tended to be seen in the west of the country. However, DT160 was concentrated in southeast England and incidents only occurred between 1993 and 1997. Interestingly, this paper showed that DT56v first appeared in garden birds in England/Wales in 1995, initially occurred only sporadically but then increased substantially in 2000 and 2001; these were the same years when DT56v was found to be endemic on a site in southwest Scotland (Pennycott* et al., 2002a). Hughes et al. (2008, 2010) used a combination of phenotyping and genotyping to characterise strains of *S. Typhimurium* recovered from wild birds from northern England. Twenty-one of 25 (84%) isolates from sparrows and finches were identified as DT56 (subsequently re-classified as DT56v by Lawson et al., 2014), three were DT40 and one was phage type (PT) U277. Multilocus sequence typing (MLST) of five of the DT56 isolates and the PT U277 isolate showed them all to be the same sequence type (ST568). An isolate of DT40 was identified as ST19, differing from ST568 in the profile of one allele only. The authors concluded that the strains of *S. Typhimurium* isolated from wild birds were host adapted and clonal. The combined results from Scotland, England and

Wales indicate that the epidemiology of salmonellosis in garden birds is likely to change over time, with the appearance and disappearance of particular phage types in different parts of the country.

4.2.3 Sex ratio

Salmonellosis appears to be recorded more frequently in male greenfinches than female greenfinches. Pennycott* et al. (1998) noted the condition in 36 males and 13 females in Scotland, and Lawson et al. (2010) diagnosed salmonellosis in 64 males and 37 females in England and Wales. The latter authors suggested a number of potential explanations, including greater numbers of males than females visiting feeding stations, greater exposure or susceptibility of males to pathogens, and increased likelihood of carcasses of male greenfinches being found due to the brighter colours of their plumage. In contrast, Grant et al. (2007) captured 136 live greenfinches over a twelve-month period, screened their faeces for salmonella, and found no significant association between the sex of a greenfinch and the likelihood of it being positive or negative for *S. Typhimurium*. In that study, *S. Typhimurium* DT56v was recovered from six live and four dead greenfinches, all in the months of January and February. Lawson et al. (2010) found no difference in the sex ratio of house sparrows with salmonellosis, possibly due to the duller plumage of both male and female birds.

4.2.4 Disease in humans, pets and livestock

In addition to infecting garden birds, these wild bird strains of *S. Typhimurium* can potentially cause disease in humans, domestic pets, horses and livestock. Horton et al. (2013) used a combination of phage typing, multilocus variable number of tandem repeats analysis (MLVA) and pulsed field gel electrophoresis (PFGE), and showed that some strains isolated from wild birds, livestock and domestic pets had up to 100% similarity, supporting the hypothesis that salmonellosis in pets and livestock could be acquired from garden birds. However, Pennycott* et al. (2006) noted that wild bird strains contributed less than 0.5% of the isolates of *S. Typhimurium* from cattle, sheep,

pigs, chickens or turkeys, but these wild bird strains were more commonly found in extensively kept domestic birds such as ducks, geese and gamebirds.

Philbey et al. (2008) described the isolation of *S. Typhimurium* DT40 and DT56v from eight cats with diarrhoea and one cat that was vomiting. All the cats had a history of catching garden birds and most became ill in the months of November to February, coinciding with the temporal pattern of salmonellosis described in garden birds. The geographic distribution of different phage types in cats also mirrored that described for garden birds. The same paper reported 47 isolations of DT40 from humans in Scotland between 2001 and 2007, and 29 human isolations of DT56v; both phage types were often recovered from children under five years of age.

Looking at a larger data set from England and Wales for 1993 to 2012, Lawson et al. (2014) noted 177 human incidents involving DT40, 237 of DT56v, and 104 of DT160. Almost half of the isolates came from infants. PFGE analysis showed that the human isolates were very similar to garden bird isolates, and temporal trends for the frequency of different phage types in humans and in garden birds were significantly positively correlated. Similarly, there was a positive geographic association between phage types found in humans and those found in garden birds. The results supported the hypothesis that humans, especially infants, can acquire certain phage types of *S. Typhimurium* from garden birds, and the authors discussed a number of different possible routes of infection.

Carriage of garden bird strains of *S. Typhimurium* by pet dogs was noted by Philbey et al. (2014), who identified DT40 in 13 canine isolates and DT56v in 8 isolates. Although isolates from 1954 to 2012 were examined, garden bird strains were only detected in the years 2002 to 2012. Horses can also be affected with salmonellosis, and Macdonald and Bell (1980) highlighted possible links with garden birds, based on their observation that the phage types most frequently found in horses were the same as those isolated from house sparrows and greenfinches.

4.2.5 Other serotypes, other countries

When the poultry-adapted serotypes *S. Gallinarum* and *S. Pullorum* were widespread in poultry flocks in the UK, these organisms were sometimes isolated from wild birds, most often house sparrows but also a goldfinch (Wilson and Macdonald, 1967), but were considered to be incidental findings representing spill-overs from infected poultry flocks.

This review has focused on garden bird salmonellosis in the UK. There are numerous papers and short communications about salmonellosis in garden birds in other parts of the world, with similar findings to those described for the UK. Many of the references are included in the papers cited above, but are excluded here for the sake of brevity.

4.3 Salmonellosis in finches, sparrows, buntings, dunnocks and tits examined at Ayr DSC 1994 to 2013 – results

4.3.1 Salmonellosis in finches, sparrows, dunnocks and tits - overview

A diagnosis of salmonellosis was made if *Salmonella* sp. was isolated from at least one organ on direct culture. Salmonellosis was recorded as the cause of death of 278 of the 1278 birds of this group (21.7% [95% CI: 19.5-24.1]): 157 greenfinches, 53 house sparrows, 50 chaffinches, 46 siskins, 23 goldfinches, 5 lesser redpolls, 4 dunnocks, 4 tree sparrows, 2 bramblings, 2 great tits, 1 bullfinch and 1 coal tit. The postmortem findings are described and discussed in 4.3.16. A presumptive diagnosis of salmonellosis, without confirmatory cultures, was made in a further 57 greenfinches and 3 chaffinches with lesions typical of the condition, submitted as part of larger confirmed salmonellosis incidents. These diagnoses of presumed salmonellosis were mostly made around seven years prior to the first diagnosis of finch trichomonosis, a condition that could be confused with salmonellosis (see Chapter 6), but have been excluded from the subsequent analyses in this chapter. In another 15 birds (8 chaffinches, 3 greenfinches, 2 house sparrows, 1 blue tit and 1 siskin), *S. Typhimurium* was isolated only through selenite enrichment and the diagnostic criteria for a diagnosis of salmonellosis were not met. A diagnosis of trichomonosis was recorded in four of these birds, trauma in two, cutaneous papilloma in one, *E. albertii* bacteraemia in one and adverse environmental conditions in one. The remaining six birds were all thin and the presence of *S. Typhimurium* may have been significant, but because it was only isolated through selenite enrichment the cases were recorded as “no diagnosis”.

For the purposes of data analysis, Scotland was divided into two regions, north and south, based on the local authority (LA) areas in which the carcasses were found (see Section 2.3 of thesis). In addition to analysing the results by carcass numbers, the data were also analysed by incidents, to remove potential bias that might occur if large numbers of carcasses were submitted from some locations. A confirmed case of salmonellosis was considered to be a new “incident” if there had been at least three

months since salmonellosis had been confirmed on that site. Charts in Sections 4.3.9 – 4.3.11 and 4.3.14, illustrating the monthly distribution of cases or incidents of salmonellosis, run from September to August rather than January to December, to show more clearly the seasonality of salmonellosis. Images where indicated can be found in Appendix X.

The results are presented as:

- Overall numbers of carcasses with salmonellosis by year and region (4.3.2)
- Salmonellosis (carcasses) by species and year (4.3.3 – 4.3.8)
- Salmonellosis (carcasses) by species, month and region (4.3.9 – 4.3.11)
- Salmonellosis (incidents) by phage type, region and year (4.3.12)
- Salmonellosis (incidents) by species, phage type and region (4.3.13)
- Salmonellosis (incidents) by month of onset (4.3.14)
- Sex and age of birds with salmonellosis (4.3.15)
- Nature of salmonellosis lesions (4.3.16)
- Antimicrobial susceptibility test results for *S. Typhimurium* (4.3.17)

4.3.2 Overall numbers of carcasses with salmonellosis

The total numbers of carcasses of finches, sparrows, tits and dunnocks in which salmonellosis was diagnosed are shown in Figures 4.1 – 4.3. The condition was diagnosed every year since 1997, peaking in 2006 and then falling. Cases were confirmed in 167 carcasses from the north of Scotland in 16 years, and in 176 carcasses from the south of Scotland in 15 years (no exact location was available for 5 carcasses). In six years between 1997 and 2004, salmonellosis accounted for over 40% of the diagnosable submissions (carcasses from which both liver and intestine were cultured) received that year (Figure 4.3). Although the peak number of cases of salmonellosis occurred in 2006, this coincided with increased submissions due to participation in the Garden Bird Health initiative and the escalation of finch trichomonosis. When the figures are expressed as a percentage of diagnosable submissions, it is apparent that the contribution made by salmonellosis declined after 2004.

Figure 4.1: Salmonellosis in finches, sparrows, dunnocks and tits. Number of carcasses, by year and region.

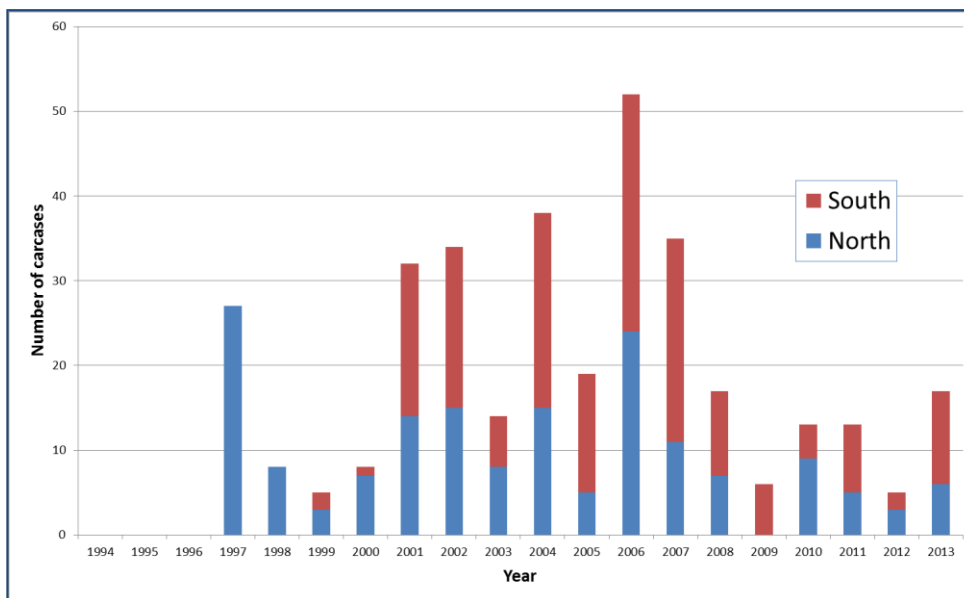


Figure 4.2: Salmonellosis in finches, sparrows, dunnocks and tits. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.

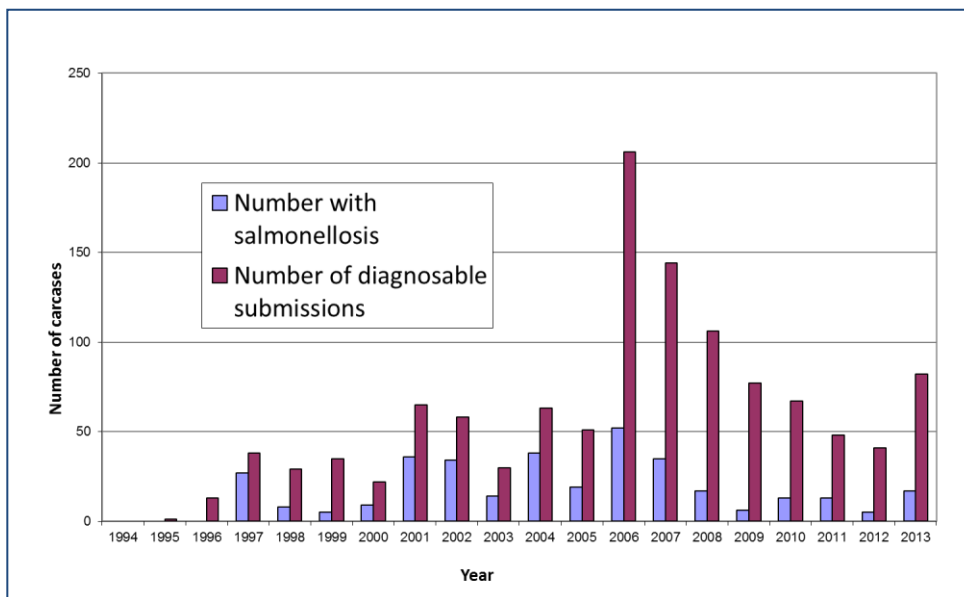
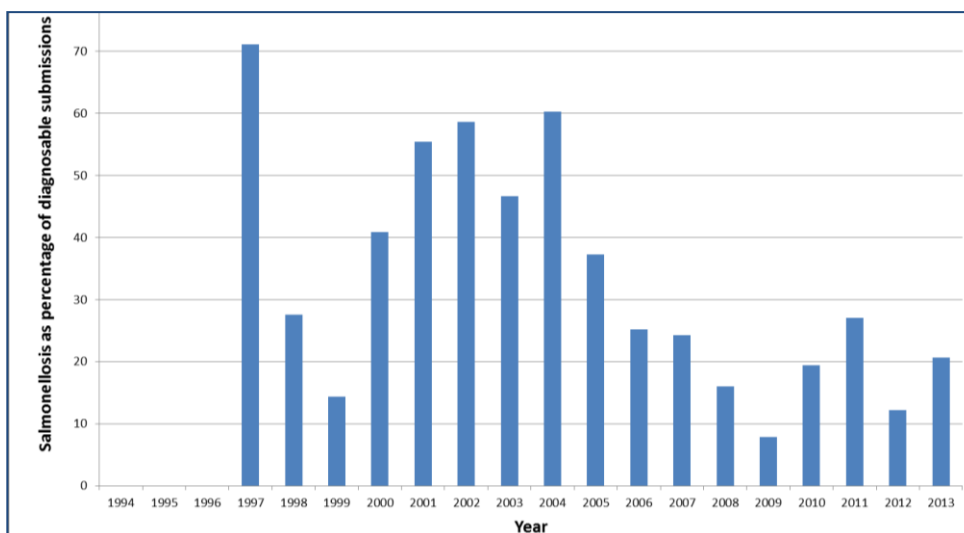


Figure 4.3: Salmonellosis in finches, sparrows, dunnocks and tits. Number of carcasses as a percentage of diagnosable submissions, by year.



4.3.3 Salmonellosis in greenfinches: by year

Figures 4.4 – 4.6 show the changing significance of salmonellosis in greenfinches. A major decline in greenfinch carcasses with salmonellosis is apparent after 2006, and when expressed as a percentage of diagnosable submissions, the decline occurred after 2004. Between 1996 and 2005, salmonellosis was confirmed in 110/167 diagnosable submissions (65.9% [95% CI: 58.1-72.9]), compared with 47/259 (18.1% [95% CI: 13.8-23.5]) between 2006 and 2013 (statistically highly significant, $p < 0.001$, two-tailed Fisher's exact test).

When expressed as the percentage of carcasses with salmonellosis that were greenfinches, the same trend can be seen (Figure 4.6): from 1997 to 2005 110/190 (57.9% [95% CI: 50.5-64.9]) birds with salmonellosis were greenfinches, falling to 47/158 (29.7% [95% CI: 22.9-37.6]) between 2006 and 2013 ($p < 0.001$, two-tailed Fisher's exact test). The comparison is even more striking for the last five years of the study; from 1997 to 2008, of 294 birds with salmonellosis, 154 were greenfinches (52.3% [95% CI: 46.5-58.2]), but from 2009 to 2013 only 3 out of 154 (1.9% [95% CI: 0.5-6.0]) were greenfinches ($p < 0.001$, two-tailed Fisher's exact test). Overall,

salmonellosis was confirmed in 36.9% (95% CI: 32.4-41.7) of greenfinch diagnosable submissions.

Figure 4.4: Salmonellosis in greenfinches. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.

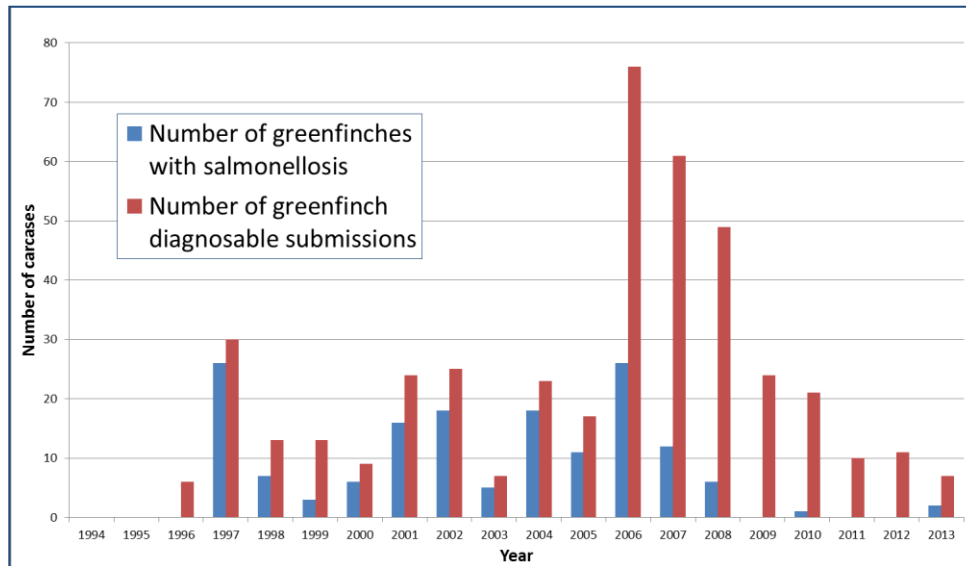


Figure 4.5: Salmonellosis in greenfinches. Percentage of diagnosable submissions in which salmonellosis was diagnosed, by year.

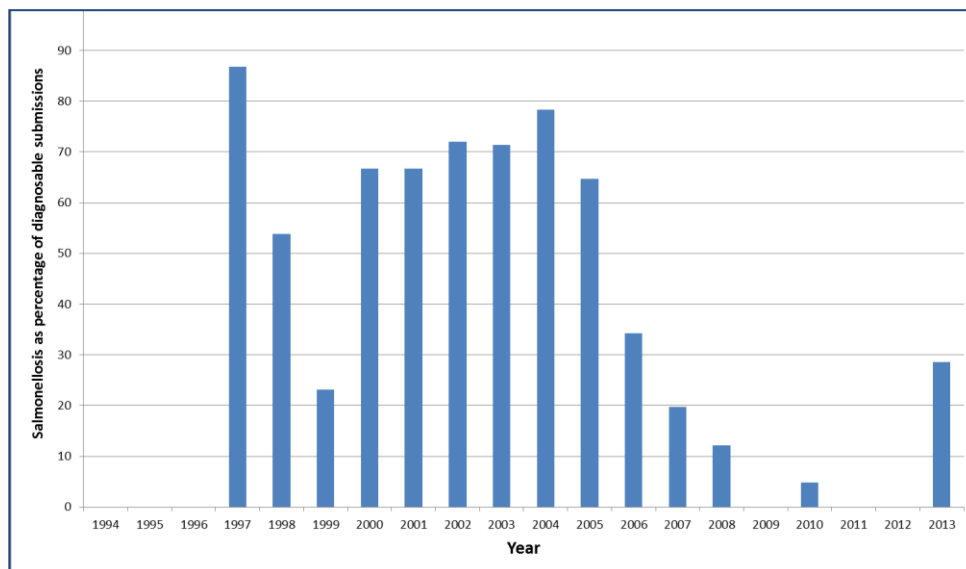
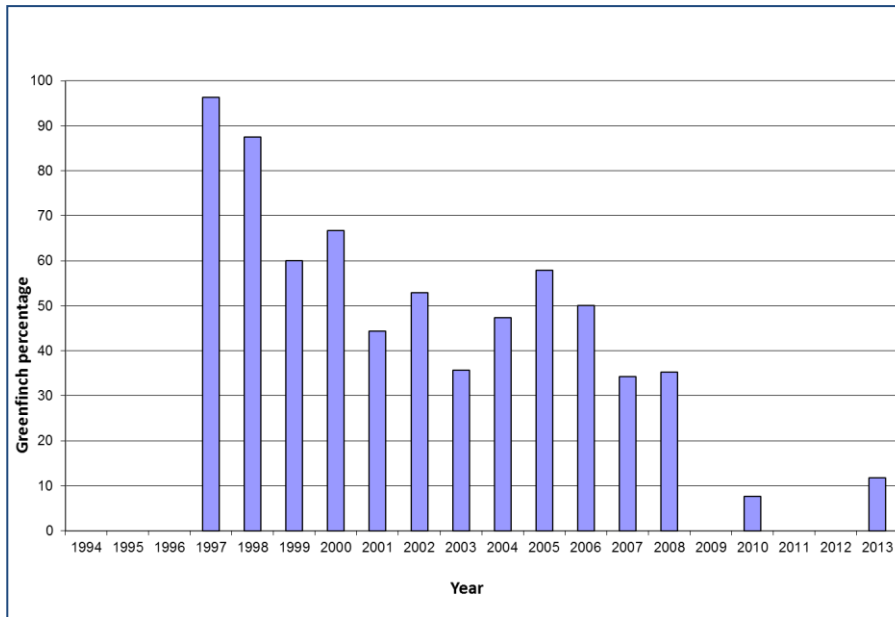


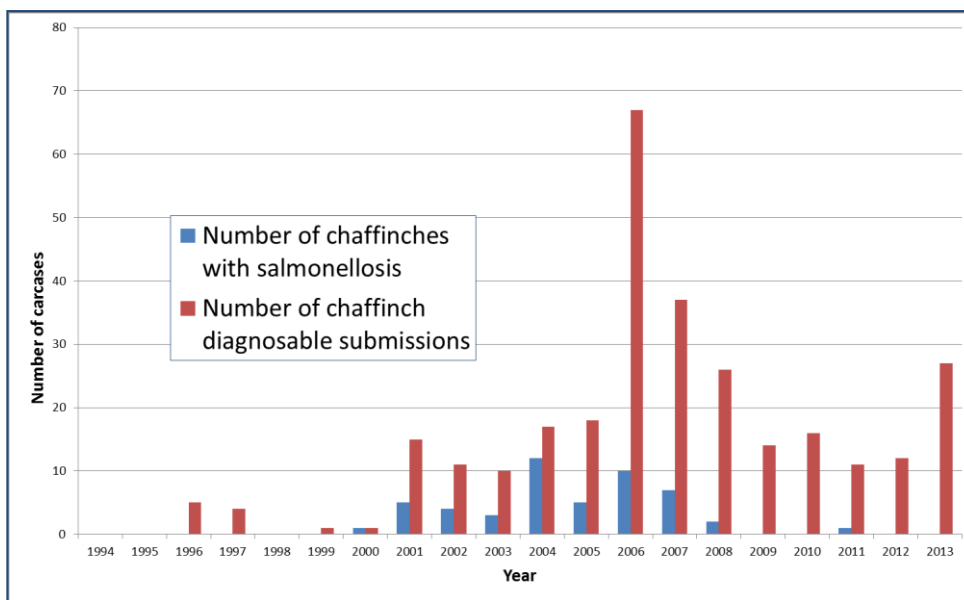
Figure 4.6: Salmonellosis in finches, sparrows, dunnocks and tits. Percentage of carcasses that were greenfinches, by year.



4.3.4 Salmonellosis in chaffinches: by year

Salmonellosis in chaffinches was more variable than in greenfinches, with most cases seen between 2001 and 2008 (Figure 4.7). From 1996 to 2008, salmonellosis was diagnosed in 49 chaffinches out of 212 diagnosable submissions (23.1% [95% CI: 17.7-29.5]), but for the period 2009 to 2013 this fell to 1/80 (1.3% [95% CI: 0.1-7.7], $p < 0.001$, two-tailed Fisher's exact test). Overall, salmonellosis was confirmed in 17.1% (95% CI: 13.1-22.0) of chaffinch diagnosable submissions.

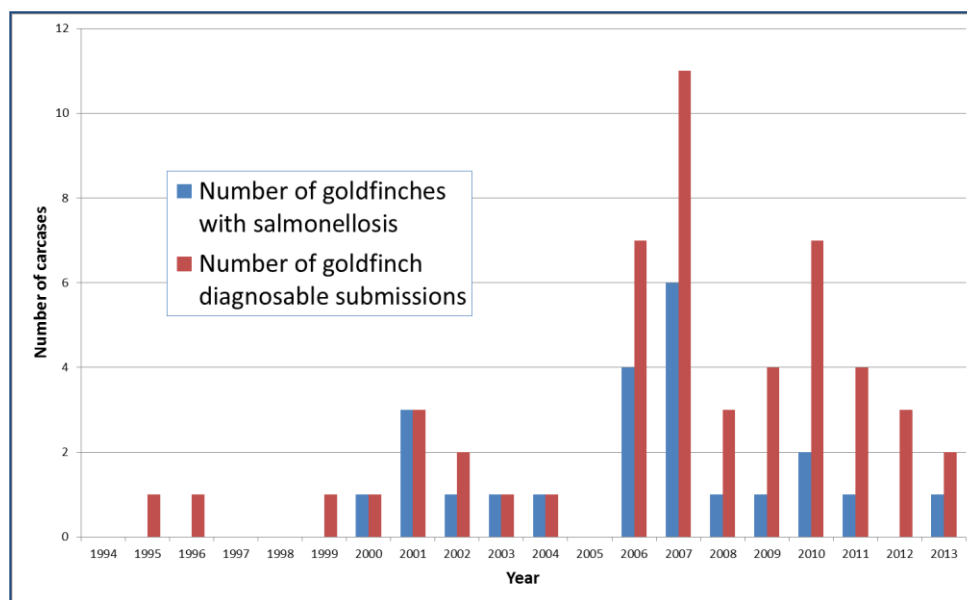
Figure 4.7: Salmonellosis in chaffinches. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.



4.3.5 Salmonellosis in goldfinches: by year

Salmonellosis was diagnosed less frequently in goldfinches than greenfinches or chaffinches (Figure 4.8), but made up a higher percentage of diagnosable submissions for this species. From 1995 to 2007, salmonellosis was diagnosed in 17 goldfinches out of 29 diagnosable submissions (58.6% [95% CI: 39.1-75.9]), falling to 6/23 for 2008 to 2013 (26.1% [95% CI: 11.1-48.7], $p=0.037$, two-tailed Fisher's exact test). Overall, salmonellosis was confirmed in 44.2% (95% CI: 30.7-58.6) of goldfinch diagnosable submissions.

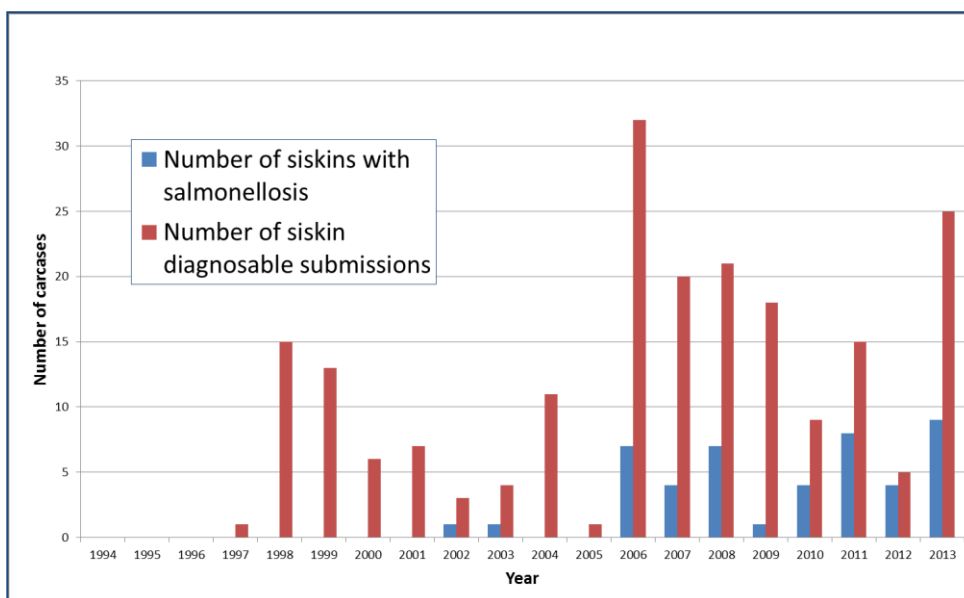
Figure 4.8: Salmonellosis in goldfinches. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.



4.3.6 Salmonellosis in siskins: by year

In contrast to the situation in greenfinches, chaffinches and goldfinches, salmonellosis in siskins was diagnosed more frequently in the second half of the study period (Figure 4.9). From 1997 to 2005, salmonellosis was diagnosed in 2 carcasses out of 61 diagnosable submissions (3.3% [95% CI: 0.6-12.4]), but between 2006 and 2013 this rose to 44/145 (30.3% [95% CI: 23.1-38.6]). This difference was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test). Overall, salmonellosis was confirmed in 22.3% (95% CI: 17.0-28.7) of siskin diagnosable submissions.

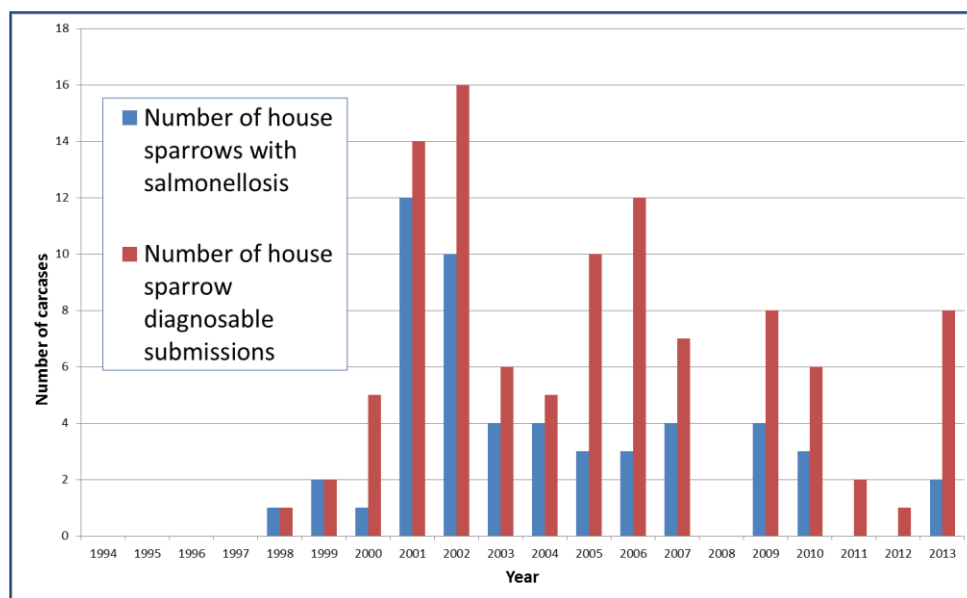
Figure 4.9: Salmonellosis in siskins. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.



4.3.7 Salmonellosis in house sparrows: by year

Salmonellosis was diagnosed in house sparrows in 13 years during the period of study (Figure 4.10), with the highest percentages of diagnosable submissions being in the first half of the study: from 1998 to 2004 salmonellosis was confirmed in 34 house sparrows out of 49 diagnosable submissions (69.4% [95% CI: 54.4-81.3]), but this fell to 19/54 (35.2% [95% CI: 23.0-49.4]) for 2005 to 2013. This difference was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test). Overall, salmonellosis was confirmed in 51.5% (95% CI: 41.5-61.3) of house sparrow diagnosable submissions.

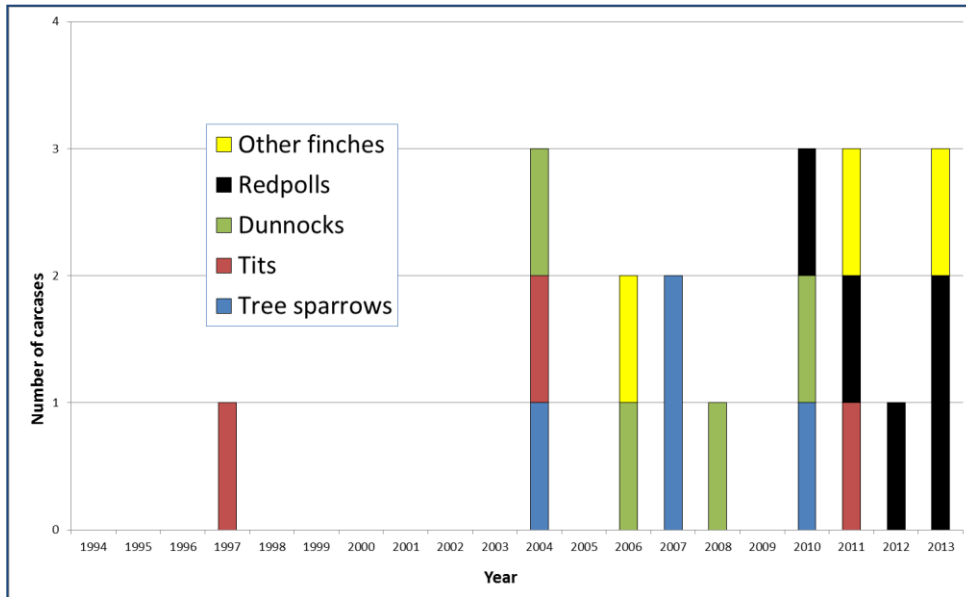
Figure 4.10: Salmonellosis in house sparrows. Number of carcasses with salmonellosis and total number of diagnosable submissions, by year.



4.3.8 Salmonellosis in other species: by year

Small numbers of other species (tree sparrows, tits, dunnocks, redpolls and other finches) died from salmonellosis (Figure 4.11). Although few in number, there was a trend for more cases to be seen in the second half of the study, and in the case of redpolls in the final four years of the study.

Figure 4.11: Salmonellosis in tree sparrows, tits, dunnocks, redpolls and other finches. Number of carcasses with salmonellosis, by year.



4.3.9 Salmonellosis in greenfinches, chaffinches and goldfinches: by month and region

The monthly distributions of salmonellosis in greenfinches, chaffinches and goldfinches are shown in Figures 4.12 – 4.14. In the north, most cases occurred in January to March (87.6% [95% CI: 79.0-93.2] of greenfinches, 100% [95% CI: 80.8-100.0] of chaffinches, and 92.3% [95% CI: 62.1-99.6] of goldfinches), with peaks in January/February. In the south the peaks occurred in January rather than February, and with fewer cases concentrated in January to March (64.9% [95% CI: 51.6-76.7] of greenfinches, 93.1% [95% CI: 75.8-98.8] of chaffinches, and 60.0% [95% CI: 27.4-86.3] of goldfinches). Few or no cases were seen in April to September (greenfinches), April to December (chaffinches) and March to November (goldfinches).

Figure 4.12: Salmonellosis in greenfinches. Number of carcasses, by month and region.

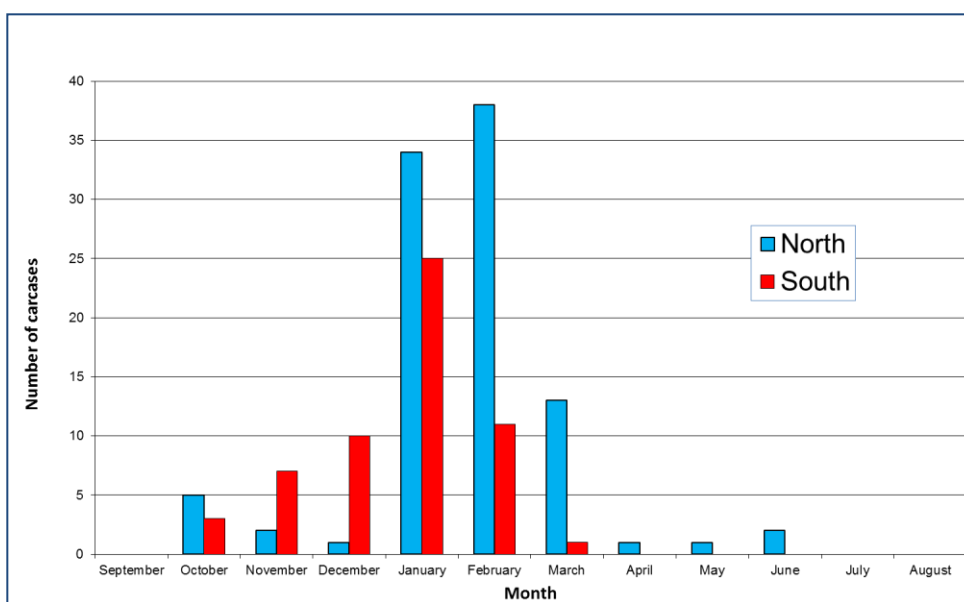


Figure 4.13: Salmonellosis in chaffinches. Number of carcasses, by month and region.

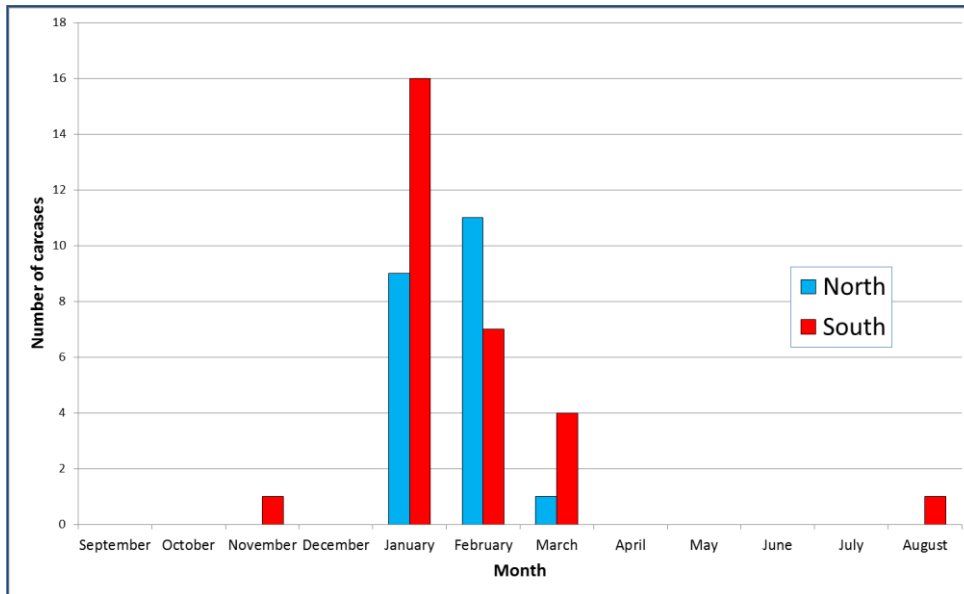
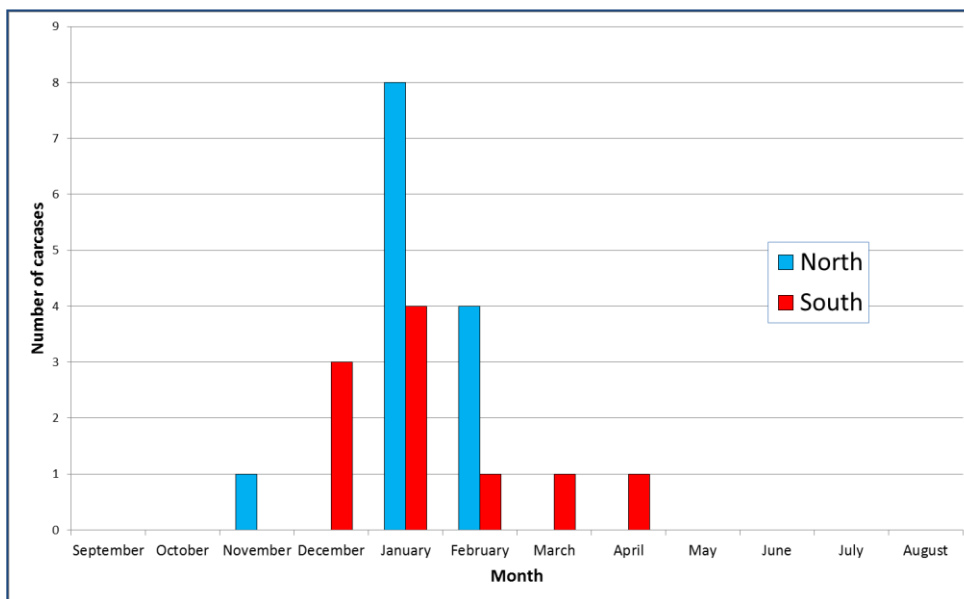


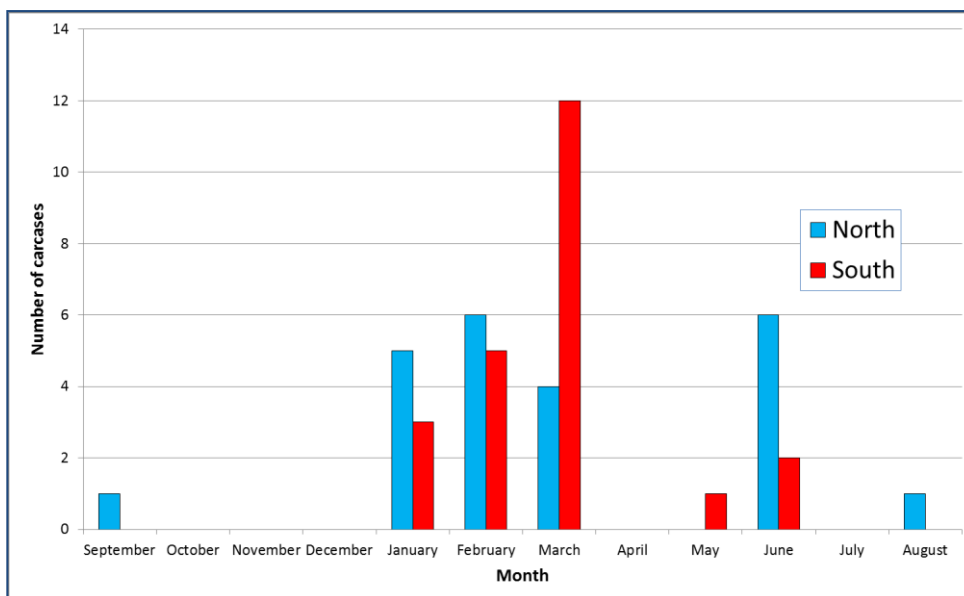
Figure 4.14: Salmonellosis in goldfinches. Number of carcasses, by month and region.



4.3.10 Salmonellosis in siskins: by month and region

The monthly distribution of cases of salmonellosis in siskins was different from the other finches (Figure 4.15). In the south, increasing numbers were seen from January to March, when 87.0% (95% CI: 65.3-96.6) of cases were seen. In the north, broadly equal numbers of cases occurred in January, February and March, overall contributing 65.2% (95% CI: 42.8-82.8) of all cases, with an additional peak of similar magnitude in June. Out-with these months, few or no cases were seen.

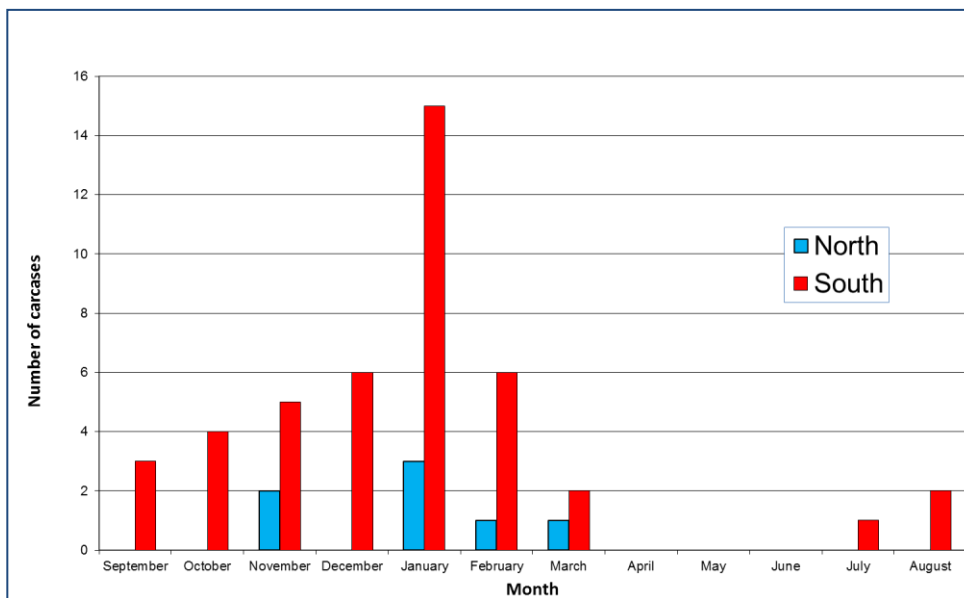
Figure 4.15: Salmonellosis in siskins. Number of carcasses, by month and region.



4.3.11 Salmonellosis in house sparrows: by month and region

The monthly pattern for house sparrows was different again (Figure 4.16). Few cases were seen in the north, where 71.4% (95% CI: 30.3-94.9) occurred in January to March. In the south numbers rose steadily from August, peaking in January and falling to March, with 52.3% (95% CI: 36.9-67.3) of cases occurring in January to March. Few or no cases were seen in the months April to July.

Figure 4.16: Salmonellosis in house sparrows. Number of carcasses, by month and region.



4.3.12 Incidents of salmonellosis: by phage type, region and year

A confirmed case of salmonellosis was considered to be a new “incident” if there was at least three months since salmonellosis had been confirmed on that site. Overall, 194 salmonellosis incidents were recorded (Table 4.1), including 100 incidents of DT40 and 83 incidents of DT56v. A phenotypic variant of DT56v reported as RDNC (reacts, does not conform) occurred on seven sites in the south of Scotland in 2011–2013, and four incidents involved more than one phage type. Precise locations were available for 189 incidents, comprising 83 in the north of Scotland and 106 in the south.

The number of incidents by year and phage type is shown in Figure 4.17 (north of Scotland) and Figure 4.18 (south of Scotland). In the north of Scotland, 90.4% (95% CI: 81.4-95.4) of incidents involved DT40, and DT56v was isolated from 9.6% (95% CI: 4.6-18.6) of incidents, but in the south the ratio was reversed, with 25.5% (95% CI: 17.7-35.0) of incidents involving DT40, and DT56v (including isolates designated RDNC) recovered from 76.4% (95% CI: 67.0-83.9) of incidents (total figure exceeds 100% because of mixed infections). After 2006 there was a reduction in the number of incidents in the north, and a similar fall in incidents occurred in the south after 2007.

Table 4.1: Incidents of salmonellosis in finches, sparrows, dunnocks and tits – by phage type and region.

Phage type of <i>S. Typhimurium</i>	Number of incidents	Location of incidents
40	100	74 in North 25 in South No location for 1 incident
56v	83	7 in North 72 in South No location for 4 incidents
RDNC (same PFGE profile as 56v)	7	7 in South
Mixed – 40 and 56v	2	1 in North 1 in South
Mixed – 40, 41, 56v	1	1 in South
Mixed – 1, 120, 208	1	1 in North

Figure 4.17: Salmonellosis in finches, sparrows, dunnocks and tits in the north of Scotland. Number of incidents, by year and phage type.

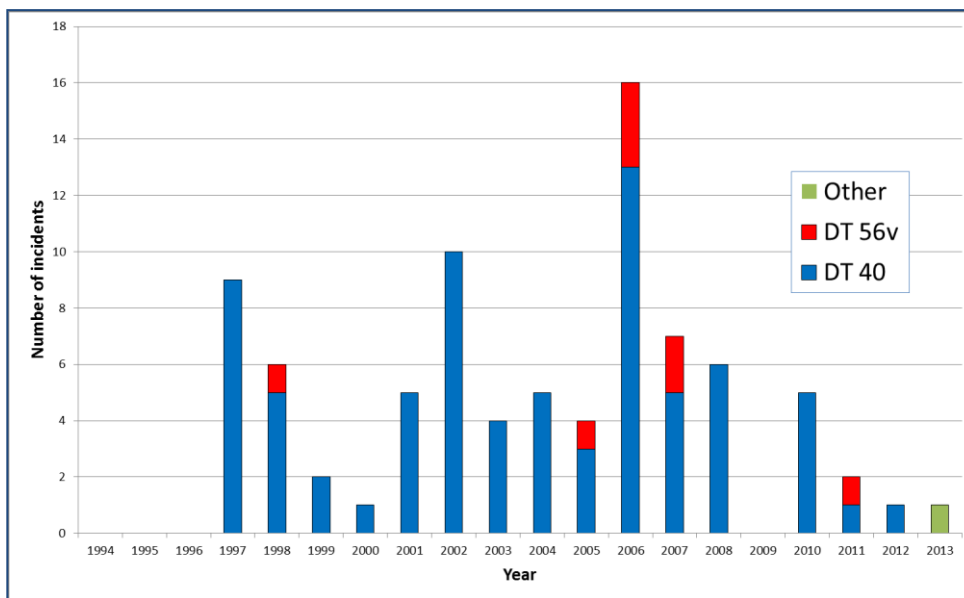
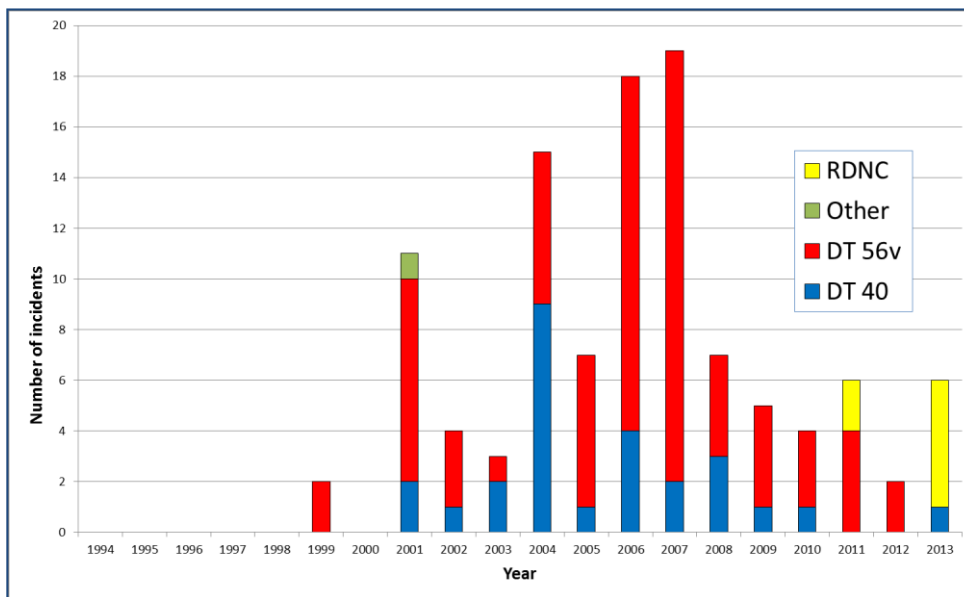


Figure 4.18: Salmonellosis in finches, sparrows, dunnocks and tits in the south of Scotland. Number of incidents, by year and phage type.



4.3.13 Incidents of salmonellosis: by species, phage type and region

Table 4.2 presents the number of incidents affecting different species of bird, by phage type and region of Scotland. As noted above, in the north of Scotland most incidents were caused by *S. Typhimurium* DT40, whereas in the south DT56v was the dominant phage type. This distribution was statistically significant for incidents involving greenfinches, chaffinches, siskins and other finches. A similar trend was seen in house sparrows but was not statistically significant, most likely reflecting the small number of incidents involving house sparrows in the north of Scotland.

Table 4.2: Incidents of salmonellosis in finches, sparrows, dunnocks and tits – by species, phage type and region.

Category of bird	Phage type of <i>S. Typhimurium</i>	Number of incidents in the north of Scotland	Number of incidents in the south of Scotland
All finches, sparrows, dunnocks and tits*	40	75	27
	56v #	8	81
Greenfinches*	40	51	12
	56v #	3	31
Chaffinches**	40	12	8
	56v#	1	14
Siskins*	40	10	3
	56v #	2	17
House sparrows***	40	5	8
	56v #	1	18
Other finches, tree sparrows, dunnocks, tits*	40	13	4
	56v #	2	19

including RDNC isolates with same PFGE profile as DT56v

*Fisher's two-tailed exact test $p < 0.001$. **Fisher's two-tailed exact test $p = 0.003$

*** Fisher's two-tailed exact test $p = 0.058$

4.3.14 Incidents of salmonellosis: month of onset

Further information on the month of onset of salmonellosis incidents is provided in Figures 4.19 – 4.23. 88.2% (95% CI: 75.4-95.1) of new incidents of DT40 in greenfinches in the north region occurred in January to March, peaking in January/February. In the south 75.0% (95% CI: 42.8-93.3) of new DT40 incidents were

recorded in these months, and none were seen in March (Figure 4.19). The picture for DT40 incidents involving chaffinches was similar, with 83.3% (95% CI: 50.9-97.1) and 87.5% (95% CI: 46.7-99.3) occurring in January to March in the north and south respectively (Figure 4.20).

Only small numbers of incidents of DT56v took place in the north and no further analysis was carried out. In the south, new incidents of DT56v in greenfinches occurred over a wider time period than DT40 in the north or south, with only 54.8% (95% CI: 36.3-72.2) of new incidents occurring in January to March (Figure 4.21). A similar percentage (50.0% [95% CI: 26.8-73.2]) of incidents of DT56v involving house sparrows occurred in January to March (Figure 4.21).

A comparison of DT40 in greenfinches in the north and DT56v in greenfinches in the south is shown in Figure 4.22, and for siskins in Figure 4.23. As noted previously, 88.2% of new incidents of DT40 in greenfinches in the north region occurred in January to March, compared with 54.8% of new incidents of DT56v in the south. No such difference was noted for siskins, with 90.0% (95% CI: 54.1-99.5) of DT40 incidents in the north being noted in January to March, and 86.7% (95% CI: 58.4-97.7) of new incidents of DT56v in the south occurring in these months, although tending to be later than in the north. There were insufficient numbers of DT40 incidents in siskins in the south or DT56v incidents in siskins in the north to permit any evaluation.

Analysis using two-tailed Fisher's exact tests showed that a significantly higher percentage of new DT40 incidents in greenfinches occurred in January to March in the north compared with DT56v incidents in the south ($p=0.002$), but no other significant associations were found.

Figure 4.19: Salmonellosis (DT40) incidents in greenfinches. Month of onset.

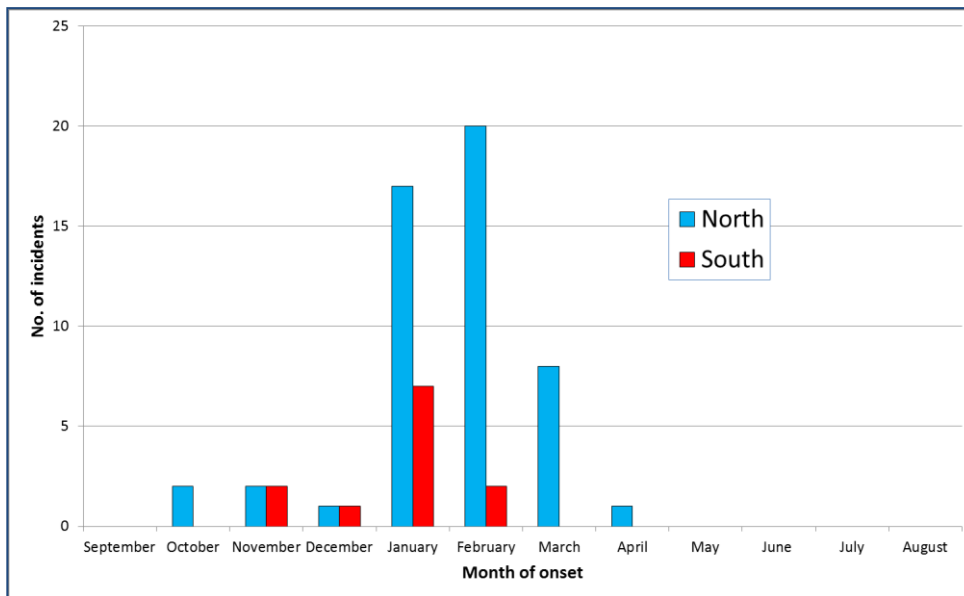


Figure 4.20: Salmonellosis (DT40) incidents in chaffinches. Month of onset.

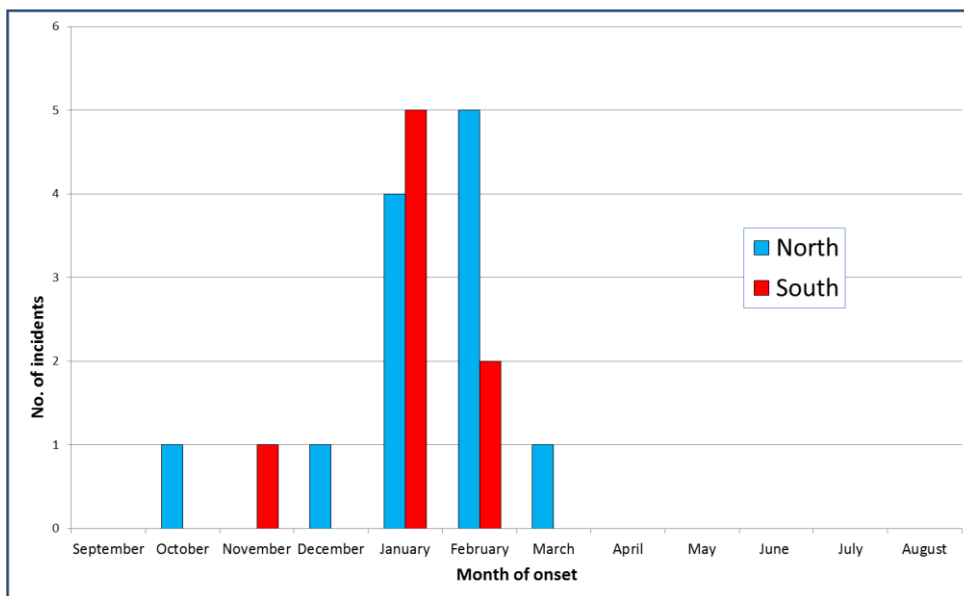


Figure 4.21: Salmonellosis (DT56v) incidents in south of Scotland. Month of onset.

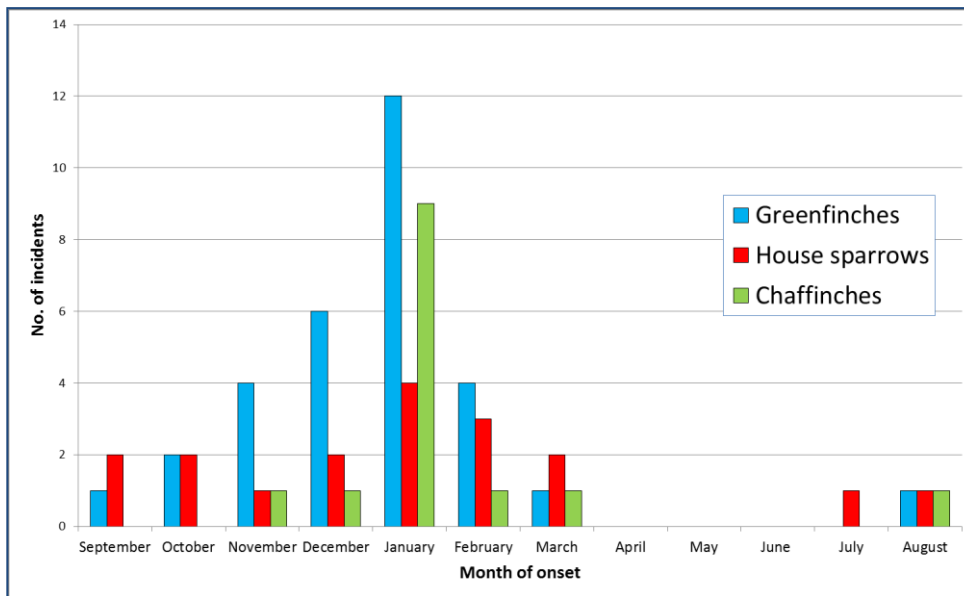


Figure 4.22: Salmonellosis (DT40 and 56v) incidents in greenfinches. Month of onset.

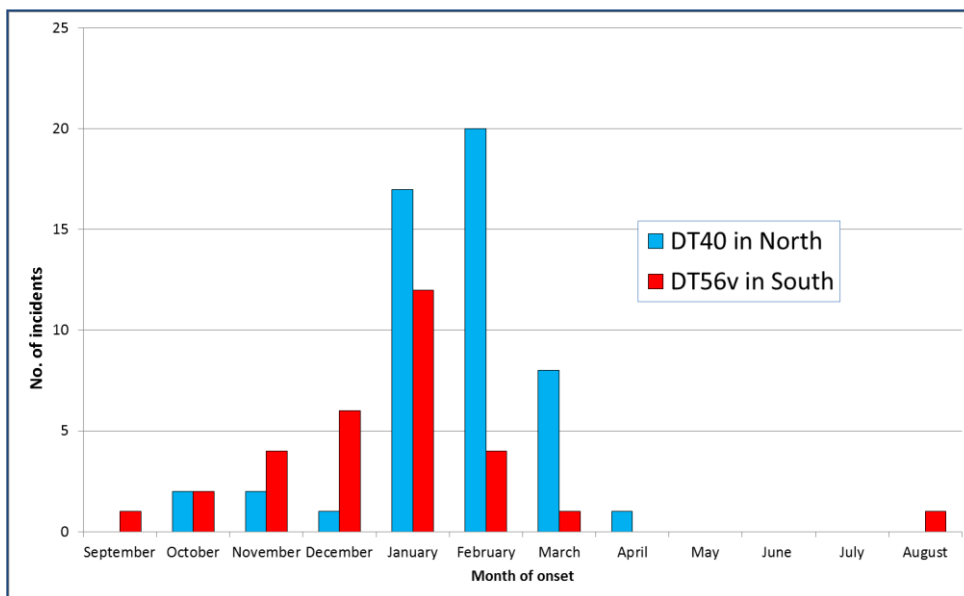
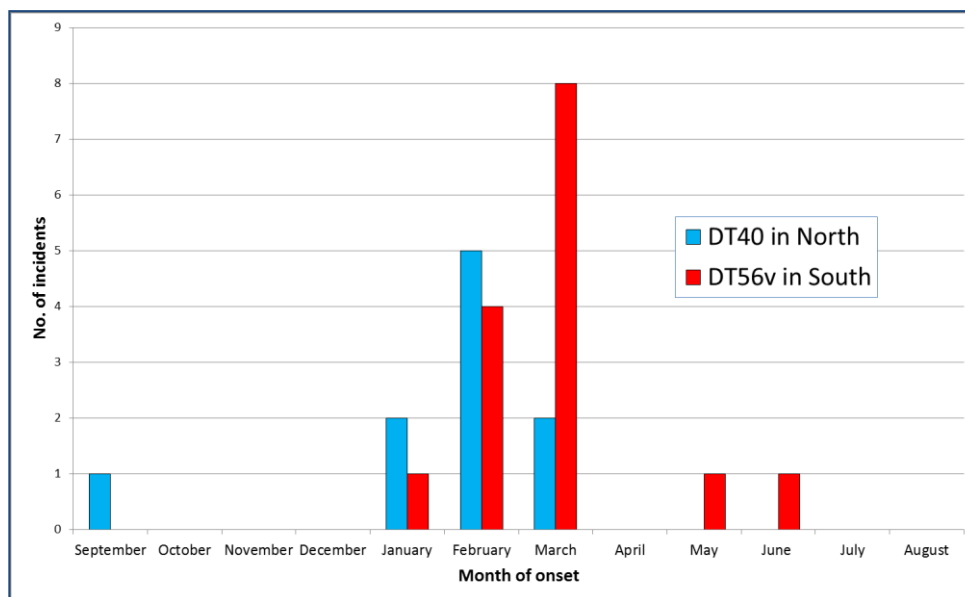


Figure 4.23: Salmonellosis (DT40 and 56v) incidents in siskins. Month of onset.



4.3.15 Sex and age of birds with salmonellosis: by month and species

Birds were categorised as immature (not yet completed their post-juvenile moult) or adult based on their plumage and the size of the bursa of Fabricius. Where possible, the sex of adult birds was established by examination of the gonads. The numbers of male birds, females and immatures with salmonellosis in different months are shown in Figures 4.24 – 4.28. Salmonellosis was diagnosed in 194 male birds and 73 female birds. Assuming a theoretical population made up of 50% males and 50% females, this difference was statistically highly significant ($p < 0.001$, two-tailed binomial test). When broken down by species, this was true for greenfinches (97M:25F, $p < 0.001$) and to a lesser extent in chaffinches (30M:13F, $p = 0.029$). The same trend was seen in goldfinches (11M:5F), siskins (25M:13F) and house sparrows (24M:14F), and although not statistically significant ($p = 0.163$, $p = 0.096$ and $p = 0.166$ respectively), the differences may be biologically significant but with insufficient statistical power.

Although the overall balance favoured male birds, variation between different months was noted. For example, male chaffinches greatly outnumbered female chaffinches in January and March, but in February the difference was less marked. In siskins, males

dominated in January and February, but in March the difference was less obvious and in June female siskins slightly outnumbered male siskins. In house sparrows, males with salmonellosis outnumbered females in December to February, but for March to November there was greater variability in the sex affected.

Salmonellosis was diagnosed in seven immature birds (2.6% of birds with salmonellosis in which the age and sex were established) – 5 greenfinches in October, 1 siskin in August, and 1 house sparrow in October. Four of the immature greenfinches and the immature siskin were found in the north of Scotland, the remaining greenfinch and the house sparrow were from the south.

Figure 4.24: Salmonellosis in finches, sparrows, dunnocks and tits. Number of carcasses of males, females and immatures, by month.

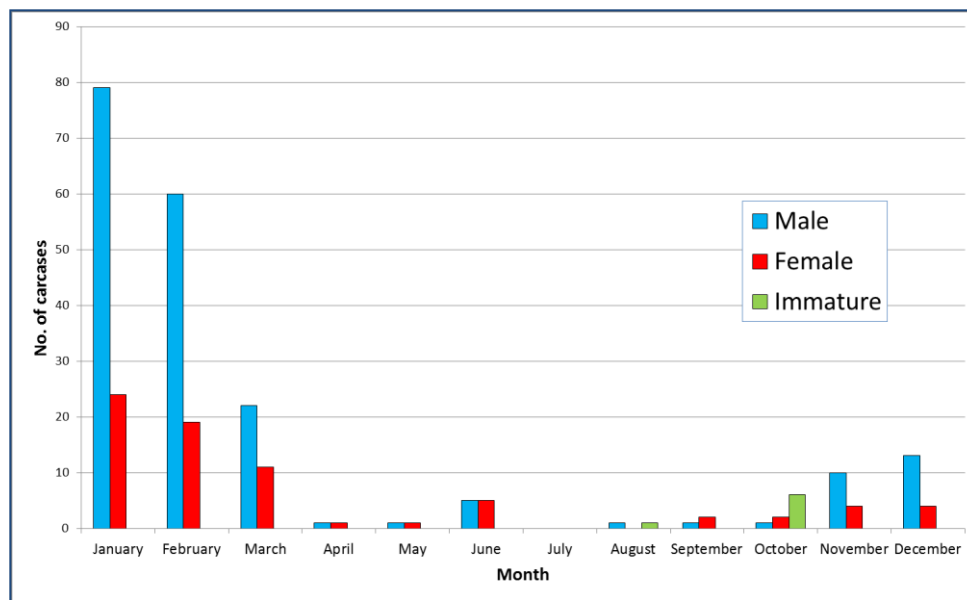


Figure 4.25: Salmonellosis in greenfinches. Number of carcasses of males, females and immatures, by month.

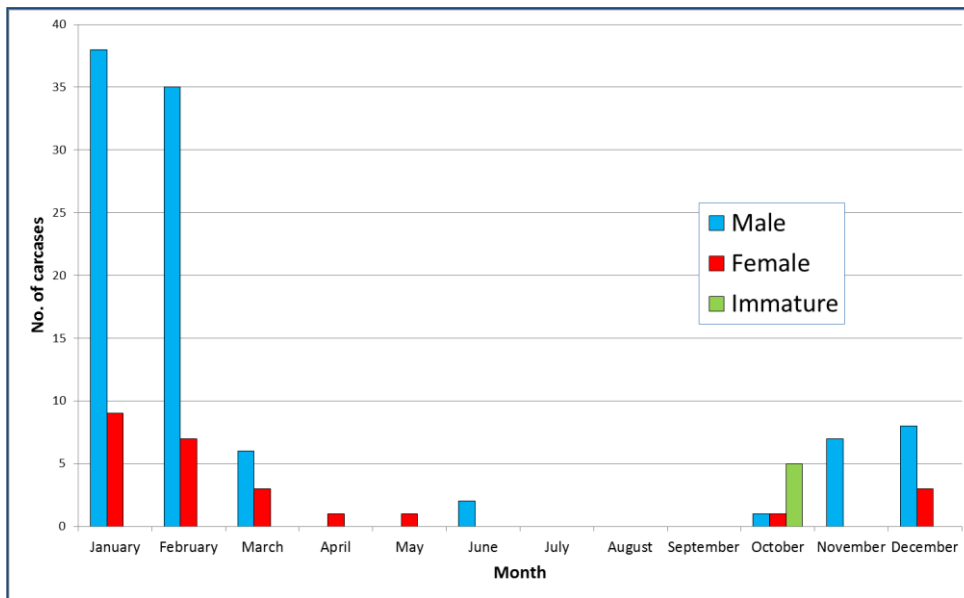


Figure 4.26: Salmonellosis in chaffinches. Number of carcasses of males, females and immatures, by month.

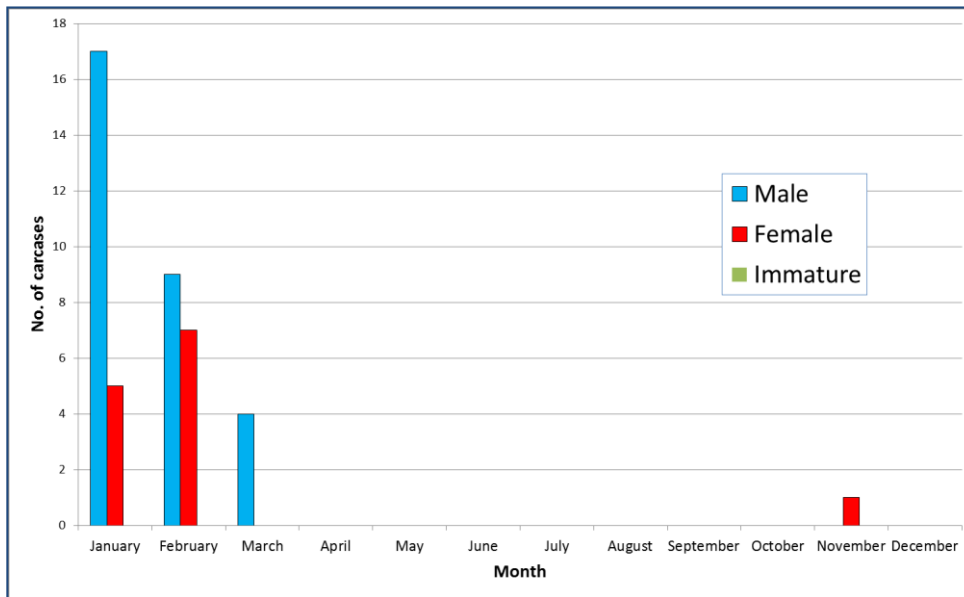


Figure 4.27: Salmonellosis in siskins. Number of carcasses of males, females and immatures, by month.

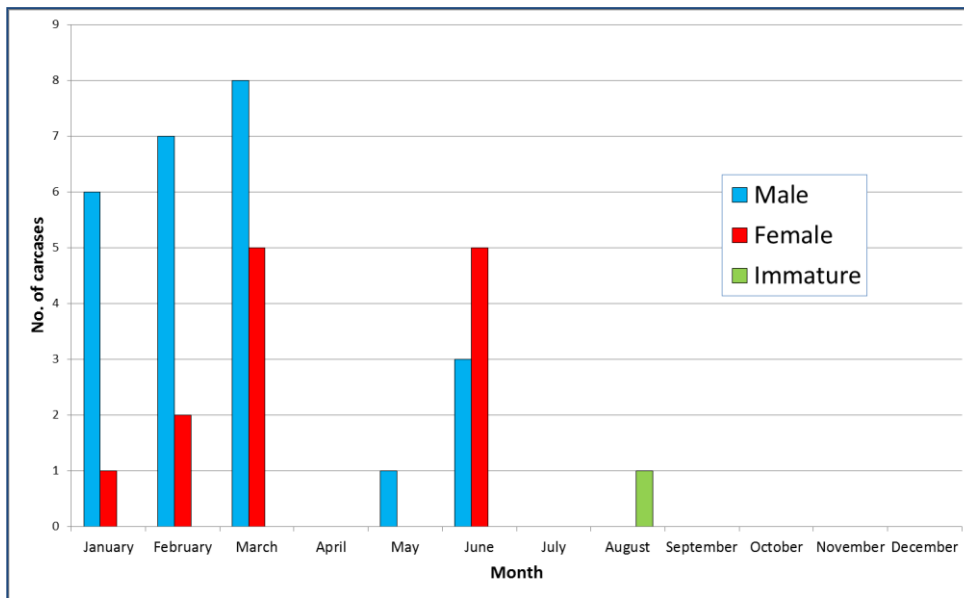
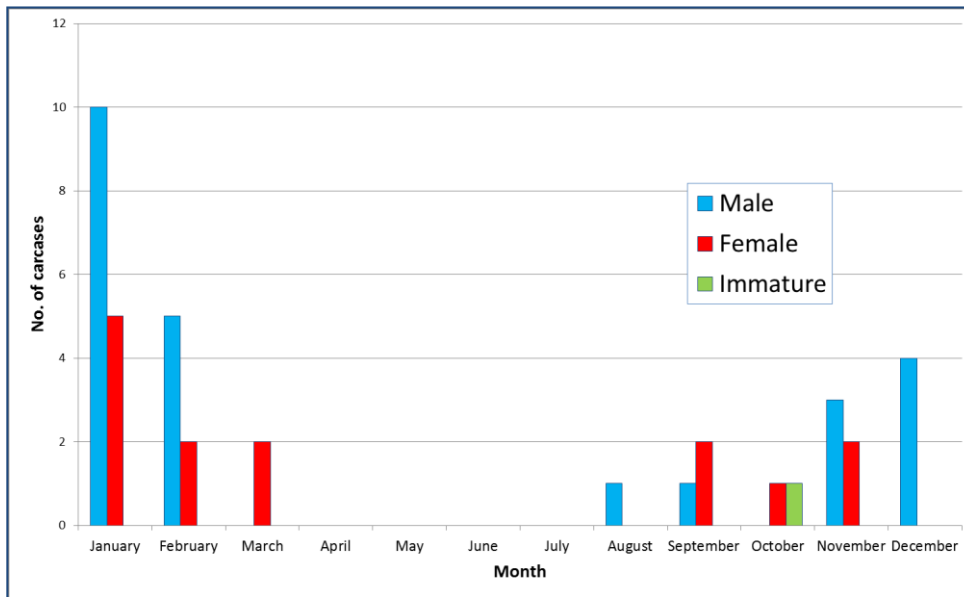


Figure 4.28: Salmonellosis in house sparrows. Number of carcasses of males, females and immatures, by month.



4.3.16 Nature of salmonellosis lesions

Postmortem lesions included focal or diffuse necrosis of the oesophagus or crop; enlargement of the liver or spleen; small necrotic foci or larger nodules in the liver, spleen, caeca, gizzard, oropharynx, rectum or cloaca; and pericarditis, perihepatitis, peritonitis, airsacculitis and shoulder joint arthritis. Images of different postmortem lesions can be found in Appendix X (**Images 39-70**). The postmortem lesions recorded for individual cases of salmonellosis were retrospectively and blindly assessed as follows:

Type 0 – no visible lesions present

Type 1 – lesions confined to digestive tract (oropharynx, oesophagus, crop, gizzard, caeca, rectum or cloaca)

Type 2 – digestive tract lesions as for Type 1 plus enlargement of liver and/or spleen, but without focal lesions

Type 3 – digestive tract lesions as for Type 1 plus necrotic foci or granulomata in liver and/or spleen

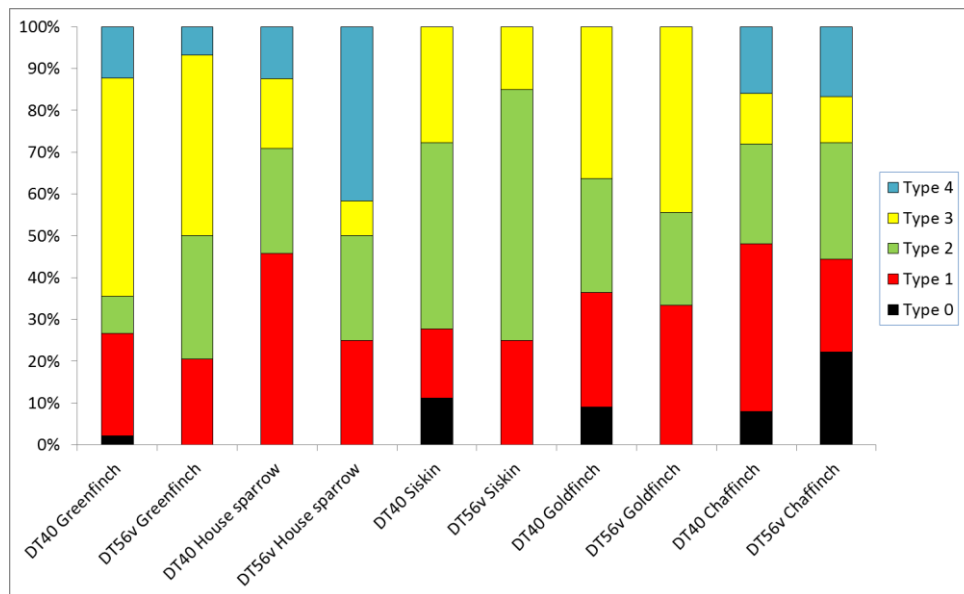
Type 4 – pericarditis, perihepatitis, pneumonia, airsacculitis, peritonitis or arthritis.

A summary of the lesions found in the species most commonly affected, by species of bird and phage type of *S. Typhimurium*, is presented in Figure 4.29. Type 3 lesions were the commonest presentation in greenfinches and goldfinches with DT40 and DT56v, but in siskins with DT40 and DT56v the commonest lesion was Type 2. Type 1 lesions predominated in house sparrows with DT40, but the commonest lesion in house sparrows with DT56v was Type 4. Chaffinches with DT40 more often had Type 1 lesions, but chaffinches with DT56v had marginally more Type 2 lesions and had the highest proportion of Type 0 lesions than other species.

Type 2 lesions were significantly more common in greenfinches with DT56v compared with greenfinches with DT40 ($p=0.006$, two-tailed Fisher's exact test), and Type 3

lesions in siskins with DT40 were significantly more common than in siskins with DT56v ($p=0.045$, two-tailed Fisher's exact test).

Figure 4.29: Salmonellosis lesions, by species and phage type.



The mean weights of birds with different types of lesion are summarised in Table 4.3. In general birds with Type 2 lesions tended to be marginally heavier than those with other types of lesions, but the differences were small, not statistically significant, and could reflect the accuracy of weighing birds in different stages of autolysis and varying levels of carcase contamination. Overall, no significant evidence was found linking body weight with the nature of the lesions.

Table 4.3: Mean weights of birds with different salmonellosis lesions

	Mean weight (g) of birds with Type 0 lesions	Mean weight (g) of birds with Type 1 lesions	Mean weight (g) of birds with Type 2 lesions	Mean weight (g) of birds with Type 3 lesions	Mean weight (g) of birds with Type 4 lesions
Greenfinches (n=120)	21.5	21.9	22.8	21.9	22.0
Chaffinches (n=41)	17.0	16.8	19.4	17.0	18.4
Goldfinches (n=21)	13.0	12.3	14.4	13.2	n/a
Siskins (n=44)	10.0	10.4	10.3	10.7	n/a
House sparrows (n=45)	16.0	21.3	22.7	22.6	21.6

4.3.17 Antimicrobial susceptibility test results

Antimicrobial susceptibility test results for 322 isolates of *S. Typhimurium* from finches and sparrows are summarised in Appendix IV. Different panels of antimicrobials were used in disc diffusion tests during the course of the study (see Chapter 2) and so not all isolates were tested against all antimicrobials. However, 32.2% of isolates tested against sulphisoxazole were resistant, and 11.8% were resistant to tetracyclines. In contrast, of 110 isolates of *S. Typhimurium* from pooled faeces collected from a bird table over a three-year-period, none were resistant to tetracyclines and only 4.5% were resistant to sulphisoxazole (Appendix IV).

4.4 Discussion

4.4.1 General comments

An overview of the analysis of the results for salmonellosis is presented here. In addition, Chapter 7 compares and contrasts the findings for salmonellosis with those for *E. albertii* bacteraemia and trichomonosis, rather than solely discuss each disease in isolation. This approach aims to help elucidate previous conundrums such as the greater number of male birds affected, the seasonality of deaths, and the evolving interactions between the different conditions in different species of birds.

4.4.2 Salmonellosis in different species of birds

The number of diagnosable submissions (carcasses from which both liver and intestine were cultured directly for *Salmonella* spp.) fluctuated during the study. From 1997 to 2005 there was an annual mean of 43 diagnosable submissions, escalating to an annual mean of 152 between 2006 and 2008 due to participation in the GBHi. This then fell again to an annual mean of 63 diagnosable submissions from 2009 to 2013. Most cases of salmonellosis occurred in greenfinches (36.9% of greenfinch diagnosable submissions), chaffinches (17.1%), goldfinches (44.2%), siskins (22.3%), house sparrows (51.5%) and dunnocks (19.0%), but only in 7.5% of diagnosable submissions from tits. In comparison, salmonellosis was not recorded as the cause of death in 158 diagnosable submissions from starlings, robins, blackbirds or thrushes (Chapter 8) or from 151 diagnosable submissions from corvids (Chapter 9), and only 5 out of 162 pigeons/doves (Chapter 11).

Possible explanations for the preponderance of salmonellosis in finches and sparrows compared with other garden birds, not only in the UK but elsewhere in the world, were suggested by Lawson et al. (2010) and included variations in innate susceptibility of some species and differences in the degree of exposure based on feeding and flocking behaviour. Given the flocking behaviour of birds such as starlings and rooks, in which salmonellosis was not detected despite testing large numbers of birds using the same

methodology as in finches and sparrows, innate susceptibility of finches and sparrows to salmonellosis seems to be the more likely explanation.

4.4.3 Decrease in the number of carcasses with salmonellosis

During the course of the study there was a marked reduction in the number of carcasses with salmonellosis. Across most species there was a reduction in absolute numbers from 2006, and when expressed as a percentage of diagnosable submissions (to take into account the increased submission rate due to participation in the GBHi from 2005 to 2008), this reduction occurred after 2004. The number of incidents of salmonellosis also declined, after 2006 (north of Scotland) and 2007 (south of Scotland). When examined by species, absolute numbers of salmonellosis in greenfinches and chaffinches declined after 2006 and in goldfinches after 2007. Absolute numbers of salmonellosis in house sparrows decreased after 2002, but the opposite trend was seen in siskins, with absolute numbers increasing from 2006. Salmonellosis as a percentage of diagnosable submissions declined after 2004 (house sparrows), 2005 (greenfinches), 2007 (goldfinches) and 2008 (chaffinches), although it increased in siskins from 2006. There was also a marked reduction in the percentage of birds with salmonellosis that were greenfinches, falling from over 50% of carcasses between 1997 and 2008 to under 2% of carcasses from 2009 to 2013. The possible reasons for this decline are discussed in Chapter 7, in the context of changing patterns of other infectious diseases such as *E. albertii* bacteraemia and trichomonosis.

4.4.4 Regional variation in phage types

In the north of Scotland DT40 predominated and was responsible for 90% of all incidents. DT40 was also present in the south of Scotland but DT56v was the commonest phage type in this region, accounting for 76% of incidents. This distribution of phage types was statistically significant for incidents involving greenfinches, chaffinches, siskins and “other finches”. The same trend was seen for house sparrows, although not statistically significant.

DT40 is a phage type that has been found in greenfinches and house sparrows in the UK for many years, and was the commonest type isolated by Lasswade Veterinary Laboratory between 1968 and 1985 (Pennycott* et al., 2010). It was also the commonest phage type recovered from garden birds in England and Wales from 1993 to 2003 (Lawson et al., 2010).

During the current study, DT56v became the commonest phage type in the south of Scotland, first appearing there in 1999. In contrast, DT56v was responsible for relatively few incidents of salmonellosis in the north, mostly from 2005-2007, although it first appeared in the north in a greenfinch in 1998 as part of an incident involving both DT40 and DT56v. Lawson et al. (2010) noted that the balance between DT40 and DT56v in England and Wales had also changed; between 1993 and 2003, DT40 was the commoner phage type, but by 2004-2007 this role had been taken over by DT56v. These authors also found that DT160 in wild birds was initially restricted to south-east England and then disappeared. The unexpected appearance and rise in importance of DT56v, and the decline of DT160, is similar to the pattern of salmonellosis in livestock such as cattle and poultry, where different serotypes and phage types appear, flourish and then disappear (Rabsch et al., 2002). These significant regional variations in the phage types of *S. Typhimurium* found in garden birds suggest that particular phage types become established in reservoir hosts that are relatively sedentary, such as greenfinches and house sparrows, with slow or limited geographic spread. In contrast, the rapid spread of trichomonosis throughout the UK and further abroad (Chapter 6) was most likely facilitated by infected chaffinches, a species that migrates large distances (Lawson et al., 2011c).

Testing by PFGE has shown that DT56v isolates tested from Scotland generated an identical PFGE pattern, one that is indistinguishable from isolates reported from Norway as PT U277 (Derek Brown, Scottish Salmonella Reference Laboratory, personal communication). PFGE also showed that the Scottish DT56v isolates were indistinguishable from DT56/DT56v isolated from other hosts including cattle, horses

and turkeys (Ernesto Liebana, AHVLA, personal communication). Hughes et al. (2008) reported that PFGE analysis of 23 isolates of DT56 from wild birds in the north of England had 99% similarity, and one isolate of PT U277 was also 99% similar. Lawson et al. (2010), looking at a larger sample size, found that 83 of 85 isolates of DT56v from garden birds in England and Wales were indistinguishable. DT56, DT56v and possibly U277 therefore appear to be members of one large widespread clone.

In the final three years of the study, eleven isolates from seven incidents in the south of Scotland were classified by SSRL as RDNC, differing slightly on the basis of phage typing from DT56 and DT56v, but having the same PFGE pattern as DT56v (Derek Brown SSRL, personal communication). The same organism was recovered from a captive-bred siskin and greenfinch from an outdoor aviary where multiple deaths occurred and which was close to a site where salmonellosis caused by *S. Typhimurium* RDNC had been diagnosed in wild house sparrows (T. Pennycott, unpublished data). It will be interesting to see if this represents yet another phenotypic change to this evolving organism, and it will be crucial in the future that a full range of phenotypic and genotypic tests are used to compare isolates from wildlife, livestock, pets and humans.

4.4.5 Seasonal patterns of salmonellosis

The seasonal patterns of garden bird salmonellosis in Scotland were discussed by Pennycott* et al. (2006) and Pennycott* et al. (2010), and these analyses are now enhanced by the examination of a larger number of results over a longer time frame. In the north of Scotland, between 85% and 100% of greenfinch, chaffinch and goldfinch carcasses with salmonellosis were submitted in January to March, but in the south greenfinch and goldfinch cases tended to be earlier and less concentrated, with 65% and 60% respectively in the months January to March. Cases in house sparrows were also earlier and less concentrated in the south than the north; 52% of cases occurred in January to March in the south, compared with 71% in the north. For siskins the reverse was true, with 65% of cases being recorded between January and March in the north, and 87% in the south. Similar trends but with additional details were seen when

analysing the data by the month of onset of salmonellosis incidents rather than the month of submission of each carcase, further sub-divided by phage type of *S. Typhimurium*. New incidents of DT40 in greenfinches and chaffinches tended to continue later in the year in the north compared to the south. There was a trend for new incidents of DT56v in siskins in the south to occur later than DT40 in siskins in the north. However, the opposite was true for greenfinches, in which new incidents of DT56v in the south tended to occur earlier than DT40 in the north. Variation in seasonal patterns was therefore sometimes seen between regions, between species of bird and between phage types. The reasons are likely to be multiple and complex, and could include factors such as when different species of birds visit gardens in different regions in Scotland and the pathological effects of different phage types of *S. Typhimurium*. The seasonal pattern of salmonellosis is further explored in Chapter 7, by comparing monthly patterns of salmonellosis with patterns of *E. albertii* bacteraemia and trichomonosis.

4.4.6 Sex and age of birds with salmonellosis

Where adult sex was confidently established, salmonellosis was diagnosed more frequently in male birds than females. This was statistically significant for greenfinches and chaffinches, and the same trend was seen in goldfinches, siskins and house sparrows. Salmonellosis was found only in small numbers of immature birds. In theory this could reflect greater susceptibility of adult male birds to salmonellosis, more frequent use of feeding stations by adult males, or ease of finding adult male carcasses if they are more brightly coloured. However, as for seasonal patterns, interpretation will be aided by comparing the sex and age of birds with salmonellosis with the patterns seen in *E. albertii* bacteraemia and trichomonosis, and this is further explored in Chapter 7.

4.4.7 Nature of lesions of salmonellosis

The nature of the lesions found in birds with salmonellosis varied between bird species and sometimes between *S. Typhimurium* phage types. Lesions confined to the digestive tract were more likely to be seen in chaffinches and house sparrows with DT40, while siskins were more likely to have additional diffuse swelling of the liver or spleen. In

contrast, necrotic foci or granulomata of the liver or spleen were commonest in greenfinches and goldfinches. House sparrows with DT56v were the species most likely to be presented with lesions such as arthritis, pericarditis, perihepatitis, peritonitis and pneumonia. These results suggest that different species of bird responded to *S. Typhimurium* in different ways; this potentially influenced factors such as the level and duration of excretion of the organisms and the likelihood of spread to other birds.

4.4.8 Antimicrobial susceptibility tests

Using the disc diffusion test, most of the isolates of *S. Typhimurium* from garden bird carcasses were sensitive to all of the antimicrobial agents against which they were tested, with the exception of sulphisoxazole and tetracyclines (Appendix IV). Over 30% of isolates from carcasses were resistant to sulphonamides and nearly 12% were resistant to tetracyclines, but tests to determine the genetic nature of the resistance were not carried out. Gilliver and others (1999) found antimicrobial resistance to be widespread in isolates of coliforms from the faeces of wild bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*), and Livermore and others (2001) reported that 8 of 20 magpies (*Pica pica*) carried Enterobacteriaceae that were resistant to one or more antimicrobials. However, the original source of the resistant bacteria recovered from the rodents and magpies was uncertain, and they may have represented a spill-over from humans or livestock into wildlife. In contrast, in the current study the isolates of *Salmonella* that were resistant to sulphisoxazole and tetracyclines were “wild bird” strains being maintained in wild birds of different species and at different locations. It is generally accepted that there is a fitness cost incurred by bacteria that express antimicrobial resistance, putting resistant bacteria at a disadvantage compared with sensitive bacteria unless antimicrobial agents are present in the environment. The persistence of antimicrobial resistant “wild bird” strains of *Salmonella* in wild bird populations is therefore surprising, and supports the view expressed by Gilliver and others (1999) that restricting the use of antimicrobials in humans and livestock may not be enough to control antimicrobial resistance. Enne and others (2001) found

sulphonamide resistance to persist in isolates of *E. coli* from humans, despite a huge decrease in sulphonamide usage in humans since 1995.

Isolates of *S. Typhimurium* showing antimicrobial resistance have been retained in the SRUC pathogen bank, and it is recommended that further testing be carried out to determine minimum inhibitory concentrations (MICs) and the genetic basis for the resistance to sulphonamides and tetracyclines.

Chapter 5

***Escherichia albertii* bacteraemia in UK finches**

5.1 Introduction

This chapter examines mortality in UK finches caused by *Escherichia albertii*, a bacterium previously referred to as *Escherichia coli* O86. Further discussion of the patterns of mortality seen with this organism is presented in Chapter 7, where comparisons between *E. albertii* bacteraemia, salmonellosis and trichomonosis are made. The Latin names of the UK birds discussed are listed in Appendix I.

5.2 Review of *Escherichia albertii* bacteraemia in UK finches

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

In April 1993, three dead male siskins were submitted to Ayr DSC, representative of a larger number of siskins found dead in the Scottish Borders (T. Pennycott, unpublished data). One bird had a large quantity of food in the oesophagus, but the remaining two birds had empty digestive tracts. Bacteriology was carried out on two of the birds and heavy pure growths of a non-lactose-fermenting (NLF) Gram-negative bacillus were recovered from intestine and liver of both birds. A commercially available identification system based on the ability of isolates to ferment different carbohydrates and decarboxylate certain amino acids (API-20E system, BioMerieux, UK) gave a profile of 5144102, identifying the organism as an NLF *E. coli*. Death was attributed to *E. coli* bacteraemia. Further deaths associated with this organism occurred in the Highland region of Scotland in 1994 and 1995, and in the Strathclyde region of Scotland in 1996 (Pennycott* et al., 1998). Siskins and greenfinches were the species most frequently involved but small numbers of chaffinches were also affected. All the deaths occurred in April or May and 35 out of 45 birds (78%) were male. Most of the birds were in good or

moderate body condition and many had accumulations of food (peanut fragments) in the upper digestive tract and gizzard, sometimes distending the oesophagus. The intestinal contents were usually dark and fluid, but no other significant gross abnormalities were detected and autolysis precluded satisfactory histopathology. As in the birds submitted in 1993, heavy growths of bacteria identified as NLF *E. coli* with the API-20E profile of 4144102 or 5144102 were isolated from small intestine and liver. Further characterisation of 42 isolates by Foster* et al. (1998) showed that all belonged to serogroup O86:K61 and had a cytopathic effect on Vero cells. More detailed examination of two isolates showed they produced cytolethal distending toxin (CLDT) but not verotoxins 1 or 2, and had the *eae* gene which is responsible for the production of attaching and effacing lesions in the intestine. However, the isolates were atypical of *E. coli* in being non-lactose fermenting or late lactose fermenting, and in giving negative biochemical reactions with sorbitol, rhamnose, sucrose and melibiose.

Additional phenotypic and genotypic characterisation of 34 isolates was reported by La Ragione* et al. (2002), who confirmed toxicity on Vero cells due to the production of CLDT by most isolates. The *eae* gene was identified as *eaeA*, encoding gamma intimin, which was considered unusual because classical enteropathogenic *E. coli* (EPEC) produce alpha or beta intimin, and mammalian *E. coli* O86 isolates are commonly positive for epsilon intimin. All had distinct surface appendages shown to be type 1 fimbriae and curli fimbriae, and most haemagglutinated guinea pig red blood cells, which is characteristic of type 1 fimbriae. Such fimbriae contribute to the adherence and long term persistence of pathogenic strains of *E. coli* at the intestinal epithelium. The organisms isolated from the finches were, therefore, well adapted to colonise, invade and persist.

Wild bird faeces and carcasses from two selected garden sites in south-west Scotland were screened for non-lactose fermenting bacteria, but *E. coli* O86 was not identified in any of the 239 samples of pooled or individual faeces collected (Pennycott* et al., 2002a). Monitoring at one site continued for another two years, during which time an

additional 189 samples of pooled faeces were tested (Pennycott* et al., 2005a). As before, the organism was not detected in any sample of pooled faeces, although it was isolated from a chaffinch that died after colliding with a window and from a greenfinch found dead on the site. The authors concluded that either *E. coli* O86 was not common in the population of birds studied, or the negative results may have reflected the absence of any selective or enrichment media for this organism and the potential for its presence to be masked by other NLF organisms such as *Salmonella enterica*.

Although referred to as *E. coli* O86 in the above papers, it was clear that the organism was not typical of *E. coli*. Around the same time, testing was being conducted on organisms recovered from the faeces of children with diarrhoea in Bangladesh (Huys et al., 2003). Initially identified by API-20E as *Hafnia alvei*, the bacteria were later shown to belong to the genus *Escherichia* and it was proposed that they represented a new species and be named *E. albertii* (Huys et al., 2003). Like the isolates from the finches, these bacteria were sorbitol and lactose non-fermenting and possessed the *eaeA* gene, but the isolates from humans were indole-negative whereas those from finches were indole-positive. Abbott et al. (2003) raised the possibility that different biotypes of *E. albertii* might exist, based on their observations of variations in the biochemical properties of some of the isolates they tested.

Confirmation that the organism recovered from mortality incidents in finches was *E. albertii* was made by Oaks et al. (2010), who investigated mortality in lesser redpoll finches in Alaska. At least 100 dead redpolls were reported between December 2004 and February 2005, during a period of prolonged cold weather. Gross postmortem findings were similar to those observed in the Scottish cases and histopathology, although limited by autolysis, indicated an acute proventriculitis and enteritis, with no evidence of septicaemia. Heavy growths of NLF bacteria with the API-20E profile of 5144102 and positive for the *eae* and CLDT genes were recovered from representative carcasses, and 16S rRNA gene sequencing confirmed the isolates to be *E. albertii*. As for the isolates from finches in Scotland, but unlike the human isolates, the *E. albertii* from finches in

Alaska were indole-positive. These authors also tested five isolates from Scottish finches and confirmed that they were also *E. albertii*, and that the deaths in Scotland and Alaska resulted from two different clonal types of *E. albertii*. In addition, Oaks et al. (2010) isolated sorbitol-fermenting strains with the API-20E profile 5144502* from the faeces of six healthy non-finch wild birds from Australia and considered that they also were strains of *E. albertii*. Similar to the non-sorbitol-fermenting strains from diseased finches (API-20E profile 5144102*), the isolates from the healthy wild birds were positive for CLDT and *eae* genes (* differences between the two API-20E profiles are underlined for clarity). CLDT and intimin-producing bacteria identified by PCR as *E. albertii* have also been isolated from healthy non-finch wild birds in Korea (Oh et al., 2011).

Differentiating *E. albertii* from other members of the Enterobacteriaceae using standard bacteriological techniques is difficult, and Ooka et al. (2012) expressed concern that some strains of *E. albertii* in humans might be wrongly identified as enterohaemorrhagic or enteropathogenic *E. coli* (EHEC or EPEC), based on the presence of the *eae* gene. Using combinations of different genotyping tests, these authors found that 26 out of 179 (14.5%) human isolates previously identified as EHEC or EPEC were in fact *E. albertii*. Two of the isolates also produced Shiga toxin subtype 2f, and Ooka et al. (2012) concluded that *E. albertii* was likely to be a major pathogen in humans. This conclusion was later substantiated when isolates from an outbreak of human gastro-enteritis in a restaurant in Japan were re-examined and the majority identified as *E. albertii* and not *E. coli* as initially thought (Ooka et al., 2013). Brandal et al. (2015) described the examination of presumptive strains of EPEC from humans in Norway, and showed that *E. albertii* was commonly present, especially in children under five years of age. They also found an isolate of *E. albertii* that produced Shiga toxin subtype 2a and was capable of causing potentially-fatal haemolytic uraemic syndrome. The source of infection for humans remained unclear, but the organism has been detected in lettuce, beef and poultry (Lindsey et al., 2015).

In summary, deaths in finches can be associated with strains of *E. albertii* with the API-20E profile 4144102 or 5144102 that do not ferment lactose or sorbitol but do ferment indole, possess the *eaeA* and *CLDT* genes, and give a positive reaction when serotyped using *E. coli* O86:K61 antiserum. Mortality most likely occurs due to enteritis and bacteraemia. Sorbitol-fermenting strains with the API-20E profile 4144502 and 5144502 were not associated with mortality in finches. The ongoing development of improved detection methods using molecular biology will clarify the role of this organism as a pathogen for wild birds and humans.

5.3 *E. albertii* bacteraemia in finches examined at Ayr DSC 1994-2013 – results

5.3.1 Infection with *E. albertii* - overview

A diagnosis of *E. albertii* bacteraemia in finches was made if heavy growths of bacteria with the API-20E profile 4144102 or 5144102 were isolated from both liver and small intestine, in the absence of evidence of other causes of death such as trichomonosis and trauma. When such organisms were recovered from non-finches, positive serotyping using *E. coli* O86:K61 antiserum was carried out and a diagnosis of *E. albertii* bacteraemia was only made if serotyping was positive. Between 1994 and 2013, cultures were made from both the liver and intestine of 2029 wild birds, including 1004 finches. *S. enterica* was isolated on direct culture from both liver and intestine of 337 birds, and it was possible that the presence of NLF *E. albertii* could have been masked by the NLF salmonellae. There were also five birds in which NLF *E. coli* were isolated but no API profile was recorded. These 342 birds were therefore excluded from the analysis. A full breakdown of the number of isolates of potentially pathogenic *E. albertii* from the remaining 1687 birds, including 730 finches, is given in Appendix VI.

Potentially pathogenic *E. albertii* was isolated from 196 birds: 174/730 finches (23.8% [95% CI: 20.8-27.1]) and 22/957 non-finches (2.3% [95% CI: 1.5-3.5]). The difference in isolation rates between finches and non-finches was highly significant ($p < 0.001$, two-tailed Fisher's exact test). One hundred and nine isolates from finches and 16 isolates

from non-finches were further tested using the O86:K61 slide agglutination test (Appendix VII); 108 of 109 finch isolates (99.1% [95% CI: 94.2-99.9]) were seropositive, compared with only 1 of 16 (6.3% [95% CI: 0.3-32.3]) non-finch isolates. The difference between finches and non-finches was highly significant ($p < 0.001$, two-tailed Fisher's exact test). These findings indicate a very strong association between pathogenic strains of *E. albertii* and birds of the finch group.

The results for 1994 – 2013 are presented as follows:

- Overall numbers of carcasses and incidents with *E. albertii* bacteraemia (5.3.2)
- Deaths (carcasses and incidents) from *E. albertii* bacteraemia, by species, region and year (5.3.3 – 5.3.4)
- *E. albertii* as a cause of mortality and as an incidental finding, by month and species (carcasses) (5.3.5)
- *E. albertii* mortality (carcasses), by month and region (5.3.6)
- *E. albertii* mortality (carcasses) as percentages of diagnosable submissions, by year (5.3.7)
- *E. albertii* mortality (incidents), by year and region (5.3.8)
- *E. albertii* mortality (incidents), by month and region (5.3.9)
- Sex and age of birds dying from *E. albertii* bacteraemia (5.3.10)
- Antimicrobial susceptibility test results for *E. albertii* (5.3.11)
- Sorbitol-fermenting strains of *E. albertii* (5.3.12)

5.3.2 Overall numbers of carcasses and incidents with *E. albertii* bacteraemia

Using the diagnostic criteria outlined above and in Appendix II, *E. albertii* bacteraemia was the cause of death of 151 out of 1091 finches examined: 108 siskins, 32 greenfinches, 8 chaffinches and 3 goldfinches (13.8% [95% CI: 11.9-16.1]). The postmortem findings (Images 71-73, Appendix X) were as described previously (Pennycott* et al., 1998). A confirmed case of *E. albertii* bacteraemia was considered to be a new “incident” if there had been at least three months since *E. albertii* bacteraemia had been confirmed on that site. Overall there were 87 incidents at 77 sites, of these 37

incidents (33 sites) were in the north of Scotland and 50 incidents (44 sites) were in the south of Scotland. One site in the north had incidents in four different years, another site in the north had incidents in two years, one site in the south in three years, and four sites in the south in two years.

The recovery of the organism was recorded as an incidental finding in 23 birds from 20 sites: a goldfinch and greenfinch with reproductive tract problems; two chaffinches and a greenfinch that died from trauma; two chaffinches found dead during prolonged cold weather; and from six chaffinches, four siskins, two greenfinches, a redpoll and a goldfinch that all had lesions of trichomonosis (see Chapter 6). In one chaffinch and one siskin, *E. albertii* was isolated from intestine only and the finding were recorded as incidental because the full diagnostic criteria were not met. However, in both cases the birds were submitted as part of confirmed ongoing *E. albertii* incidents, so isolation of *E. albertii* was likely to be significant. Potentially pathogenic *E. albertii* that was O86:K61 seropositive was only isolated from one non-finch species (an incidental finding in a thin barn owl that died during adverse weather).

5.3.3 Deaths (carcasses and incidents) from *E. albertii* bacteraemia: by species and region

Death from *E. albertii* bacteraemia was a significant cause of mortality of finches from both regions of Scotland, especially in siskins and to a lesser extent in greenfinches. Further details about *E. albertii* deaths and incidents in the north and south of Scotland are summarised in Tables 5.1 and 5.2. A new “siskin mortality incident” was recorded on the submission of one or more siskin carcasses from one location, if no other siskin carcasses had been submitted from that site in the preceding three months. Equivalent criteria were used to define a “greenfinch mortality incident”.

Table 5.1: Mortality (number, percentage, 95% confidence intervals) from *E. albertii* bacteraemia in different regions of Scotland - carcasses.

	North	South
Finch carcasses examined*	432	639
Finch carcasses examined in which <i>E. albertii</i> bacteraemia was the cause of death	74 (17.1% [13.8-21.1])	77 (12.1% [9.7-14.9])
Siskin carcasses examined in which <i>E. albertii</i> bacteraemia was the cause of death	55 (57.3% [46.8-67.2])	53 (47.3% [37.9-57.0])
Greenfinch carcasses examined in which <i>E. albertii</i> bacteraemia was the cause of death	15 (6.9% [4.0-11.3])	17 (6.6% [4.0-10.5])
Finch carcasses with <i>E. albertii</i> bacteraemia that were siskins	74.3% [62.6-83.5]	68.8% [57.1-78.6]
Finch carcasses with <i>E. albertii</i> bacteraemia that were greenfinches	20.3% [12.1-31.5]	22.1% [13.7-33.2]

*locations not available for 20 carcasses

Table 5.2: Mortality (number, percentage, 95% confidence intervals) from *E. albertii* bacteraemia in different regions of Scotland - incidents.

	North	South
Incidents of <i>E. albertii</i> bacteraemia	37 (33 sites)	50 (44 sites)
<i>E. albertii</i> bacteraemia incidents that involved siskins	28 (75.7% [58.4-87.6])	36 (72.0% [57.3-83.3])
<i>E. albertii</i> bacteraemia incidents that involved greenfinches	11 (29.7% [16.4-47.2])	14 (28.0% [16.7-42.7])
Siskin mortality incidents attributed to <i>E. albertii</i> bacteraemia	28 (56.0% [41.3-69.7])	36 (43.9% [33.1-55.3])
Greenfinch mortality incidents attributed to <i>E. albertii</i> bacteraemia	11 (12.4% [6.6-21.4])	14 (7.3% [4.2-12.1])

5.3.4 Deaths from *E. albertii* bacteraemia: by species, region and year

Figures 5.1 and 5.2 show the number of carcasses in each year of the study in which *E. albertii* bacteraemia was diagnosed. The condition was seen in 15 out of the 20 years of the study. Siskins were affected in 14 years, greenfinches in 12 years, and “others” (chaffinches and goldfinches) in five years. Cases were seen in the north of Scotland in 12 years and the south of Scotland in 14 years.

Figure 5.1: *E. albertii* bacteraemia. Number of carcasses, by year and species.

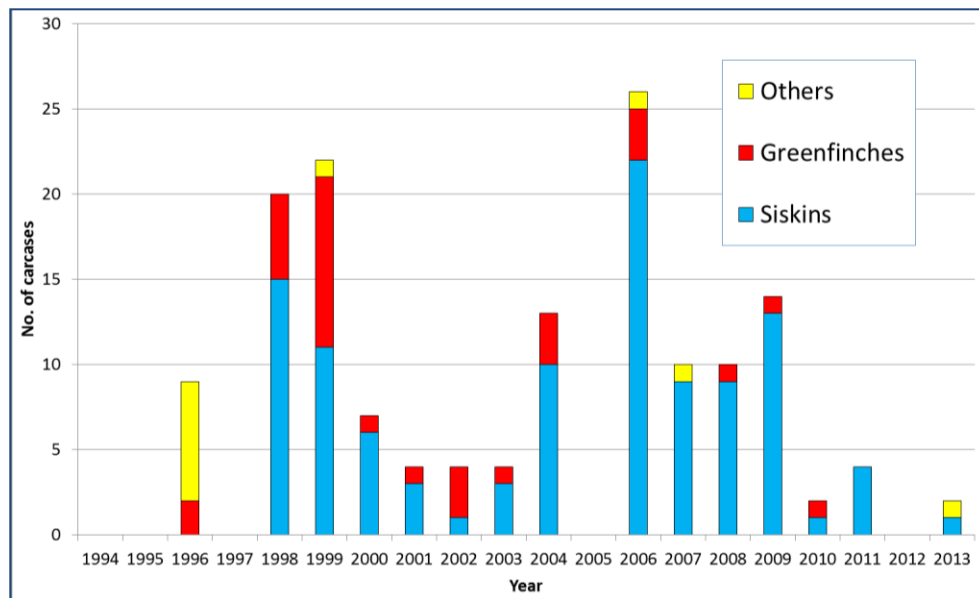
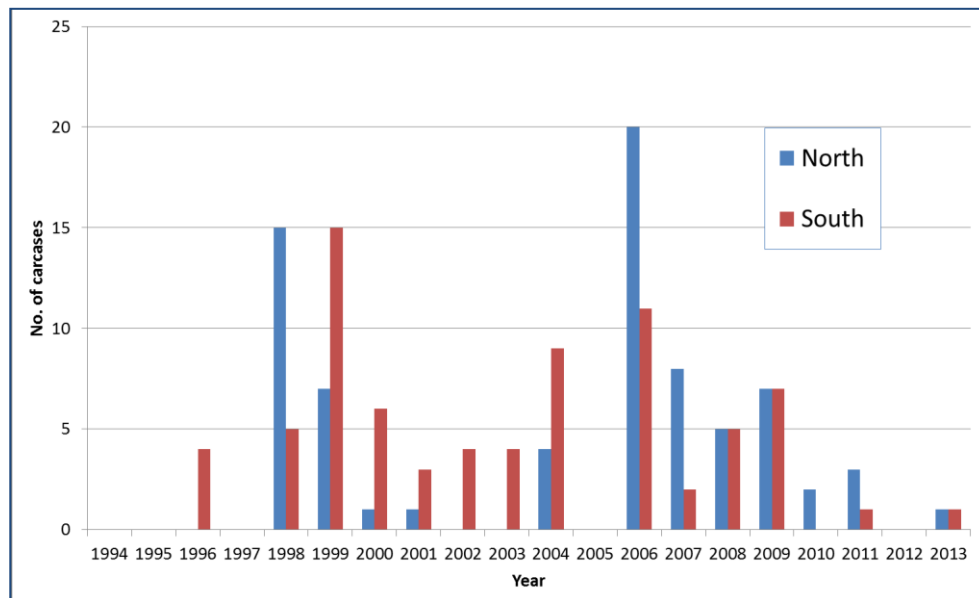


Figure 5.2: *E. albertii* bacteraemia. Number of carcasses, by year and region.



Figures 5.3 and 5.4 show the proportions of different finch species in which *E. albertii* bacteraemia was diagnosed. In 1994-2003, 60.0% (95% CI: 47.1-71.7) of cases were diagnosed in siskins and 35.4% (95% CI: 24.2-48.3) in greenfinches, but in 2004-2013

80.2% (95% CI: 70.0-87.7) of cases occurred in siskins and only 10.5% (95% CI: 5.2-19.4) in greenfinches. The difference between the two time periods in the proportions of cases diagnosed in greenfinches and siskins was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test).

Figure 5.3: *E. albertii* bacteraemia. Breakdown by finch species 1994-2003.



Figure 5.4: *E. albertii* bacteraemia. Breakdown by finch species 2004-2013.



5.3.5 *E. albertii* as a cause of mortality and as an incidental finding: by month and species

Figure 5.5 shows the monthly distribution of deaths from *E. albertii* bacteraemia, and Figure 5.6 the monthly distribution of isolations of *E. albertii* as incidental findings. Most deaths (84.6% [95% CI: 77.9-89.6]) from *E. albertii* bacteraemia occurred in April to July, but the recovery of the organism as an incidental finding was more evenly spread throughout the year.

Figure 5.5: *E. albertii* bacteraemia. Number of carcasses, by month and species.

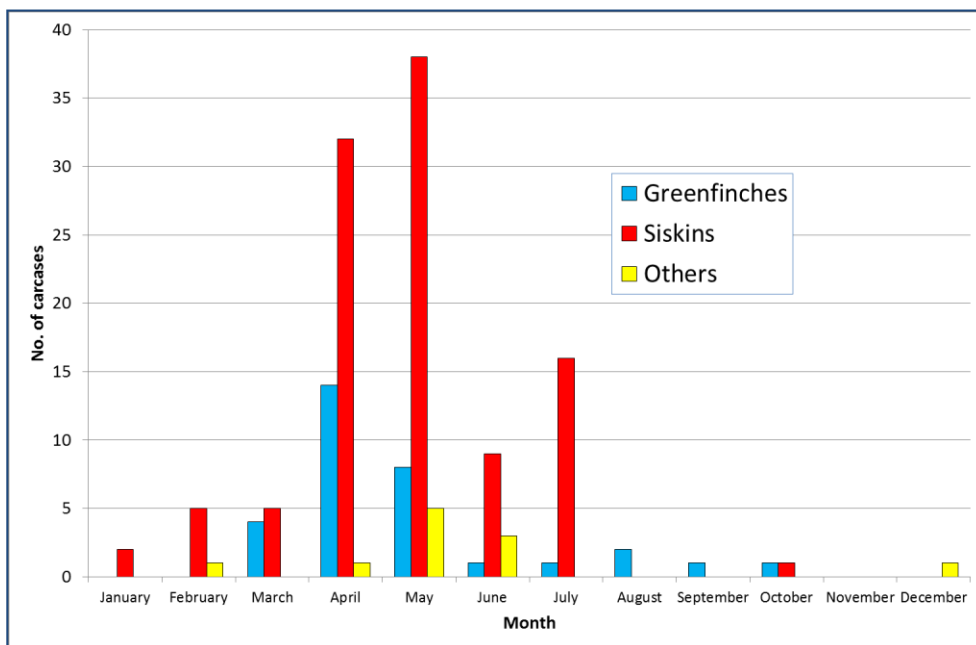
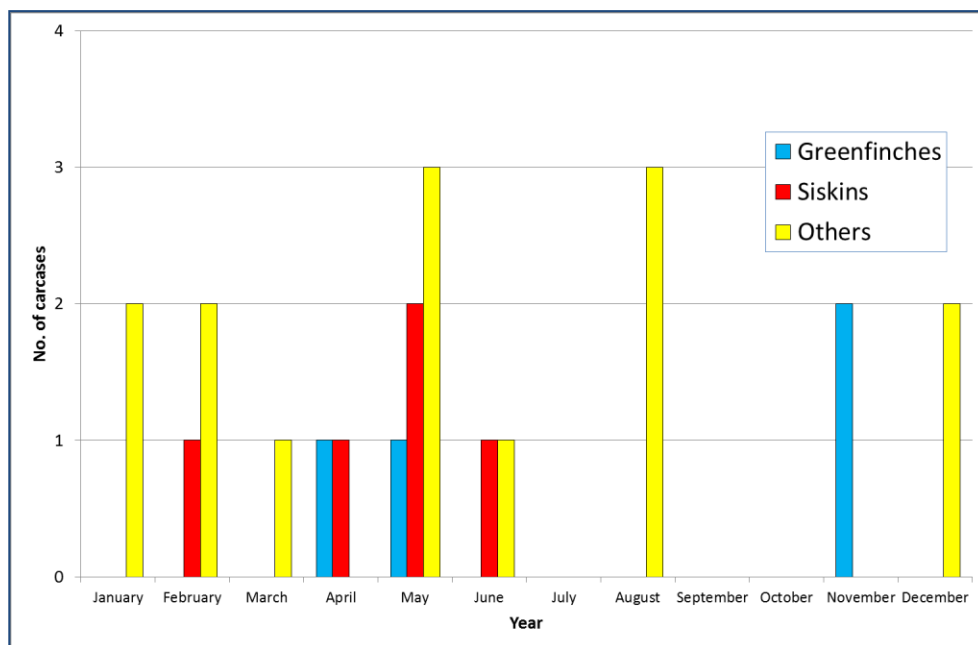


Figure 5.6: Incidental isolations of *E. albertii*. Number of carcasses, by month and species.



5.3.6 *E. albertii* mortality in siskins and greenfinches: by month and region

Further breakdowns of deaths from *E. albertii* in siskins and greenfinches in the north and south of Scotland are presented in Figures 5.7 – 5.10. Variation between region and species was noted. Most deaths in siskins in the north of Scotland were recorded in April to July, peaking in May and July, but in the south of Scotland cases tended to be seen earlier, with a peak in April/May and fewer cases in June and July (Figure 5.7). Greenfinch deaths from *E. albertii* bacteraemia peaked in April and May in the north of Scotland, and in March to May in the south of Scotland (Figure 5.8). When comparing deaths in the north of Scotland, siskin carcasses were mostly received in April to July, peaking in May and July, but greenfinch carcasses peaked in April/May and then declined (Figure 5.9). In the south, siskin carcass numbers were mostly in January to July, peaking in April/May, but greenfinch carcasses were March to May and August to October (Figure 5.10). Differences in temporal mortality patterns between the two species were therefore apparent in both regions.

Figure 5.7: *E. albertii* bacteraemia. Number of siskin carcasses, by month and region.

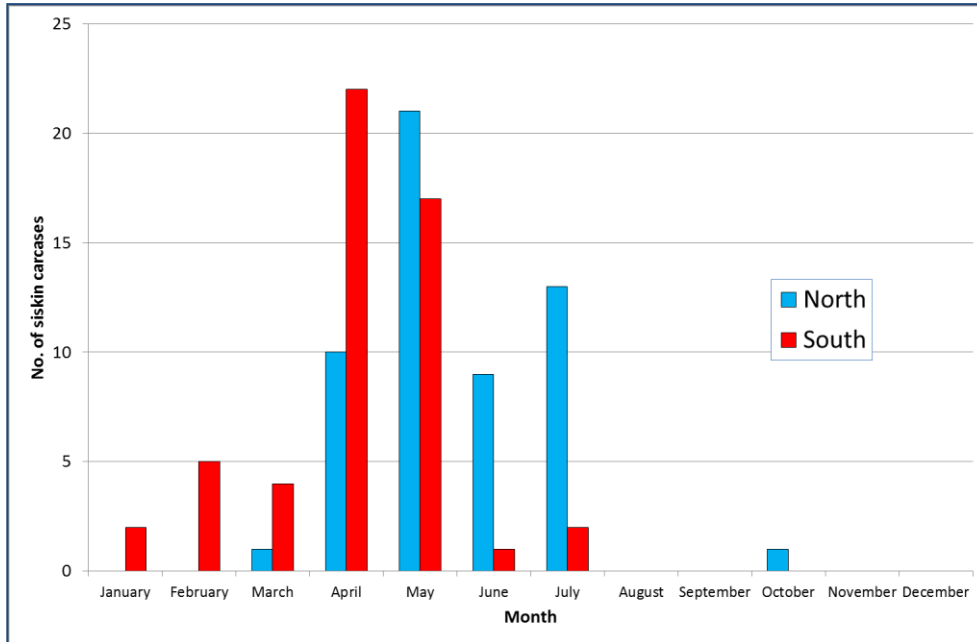


Figure 5.8: *E. albertii* bacteraemia. Number of greenfinch carcasses, by month and region.

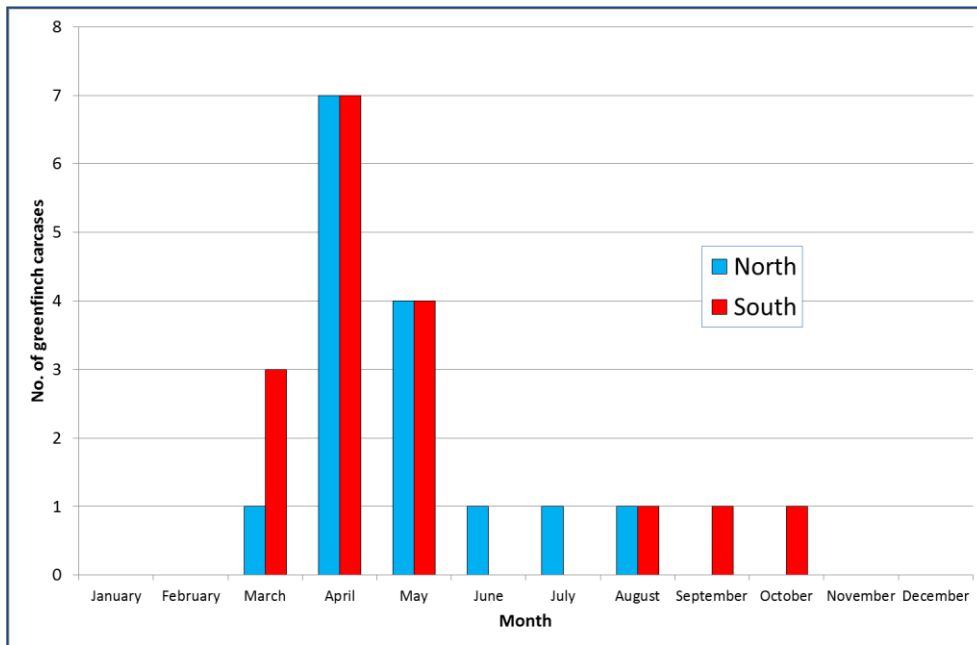


Figure 5.9: *E. albertii* bacteraemia in the north of Scotland. Number of siskin and greenfinch carcasses, by month and species.

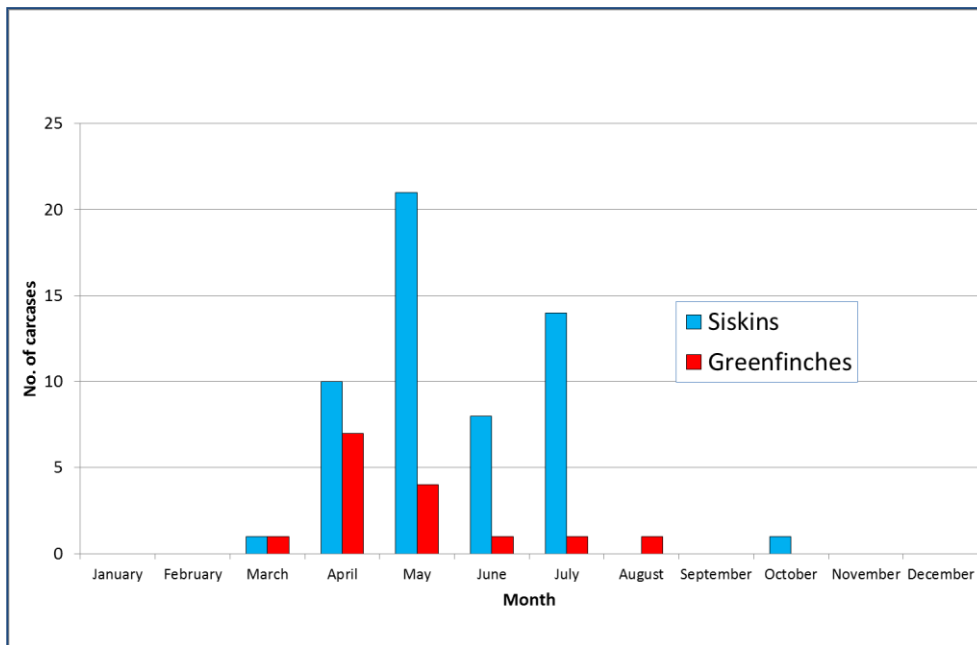
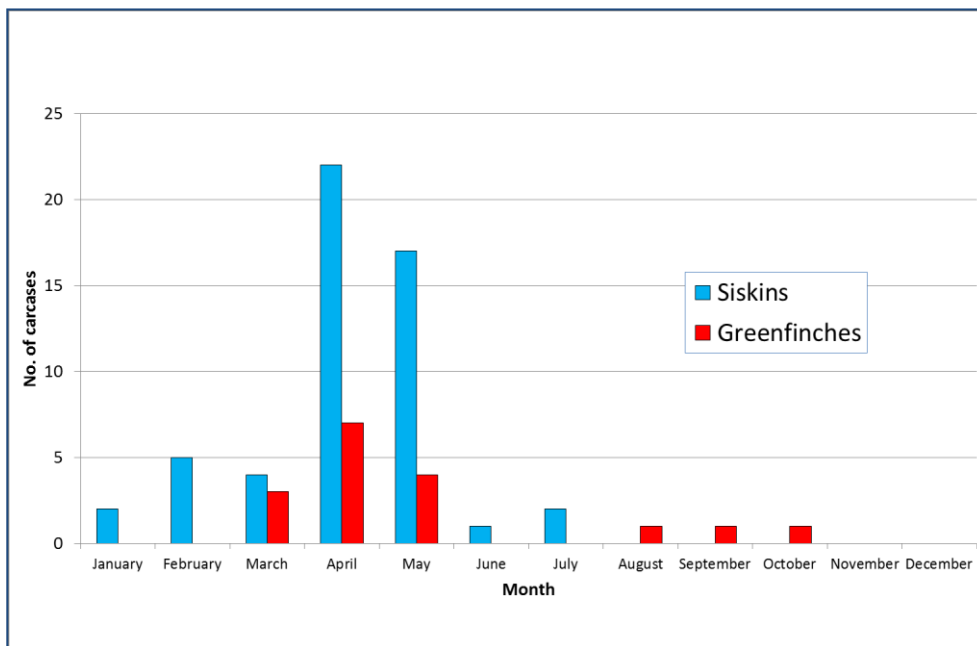


Figure 5.10: *E. albertii* bacteraemia in the south of Scotland. Number of siskin and greenfinch carcasses, by month and species.



5.3.7 *E. albertii* bacteraemia in siskins and greenfinches: as percentages of diagnosable submissions

Figure 5.11 presents the diagnoses of *E. albertii* bacteraemia in siskins as a function of the number of diagnosable submissions of siskins, i.e. those siskin carcasses from which both liver and intestine were cultured. During most of the study period, *E. albertii* bacteraemia was the commonest cause of death recorded in siskins, accounting for 102/152 (67.1% [95% CI: 59.0-74.4]) of all siskin diagnosable submissions between 1997 and 2009. However, from 2010 to 2013 this fell to 6/54 (11.1% [95% CI: 4.6-23.3]) of diagnosable submissions. The difference between the two time periods was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test). Figure 5.12 presents similar data for greenfinches: *E. albertii* bacteraemia was seen less frequently in greenfinches, and accounted for 31/377 (8.2% [95% CI: 5.7-11.6]) of all greenfinch diagnosable submissions between 1996 and 2009, reducing to 1/49 (2.0% [95% CI: 0.1-12.2]) from 2010 to 2013 ($p = 0.189$, two-tailed Fisher's exact test, not statistically significant).

Figure 5.11: *E. albertii* bacteraemia in siskins. Number of carcasses with *E. albertii* bacteraemia and total number of diagnosable submissions, by year.

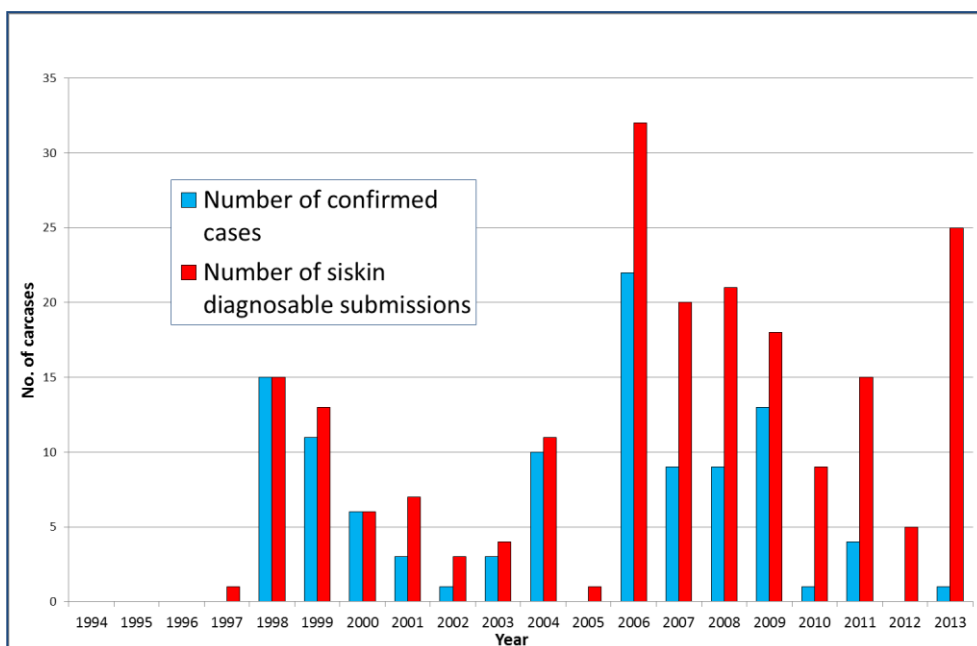
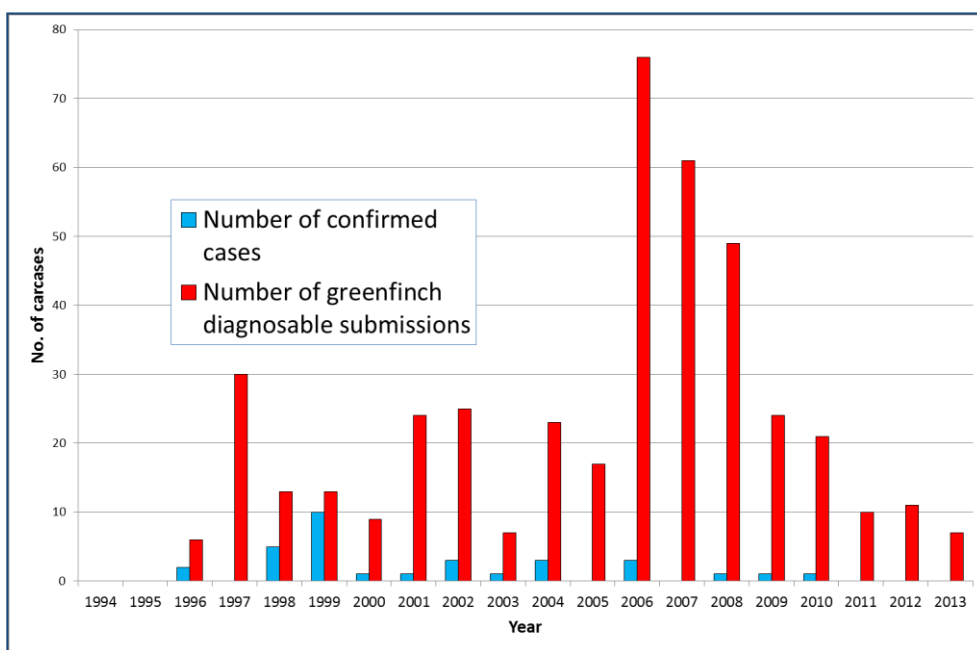


Figure 5.12: *E. albertii* bacteraemia in greenfinches. Number of carcasses with *E. albertii* bacteraemia and total number of diagnosable submissions, by year.



5.3.8 Deaths from *E. albertii* bacteraemia: number of incidents by year and region

Analysis of the data by the number of incidents rather than the number of carcasses is shown in Figures 5.13 – 5.14. There was considerable variation in the number of incidents recorded in each region each year, but few incidents were recorded in the final four years of the study. This reduction was observed in both regions.

Figure 5.13: *E. albertii* bacteraemia. Number of incidents, by year.

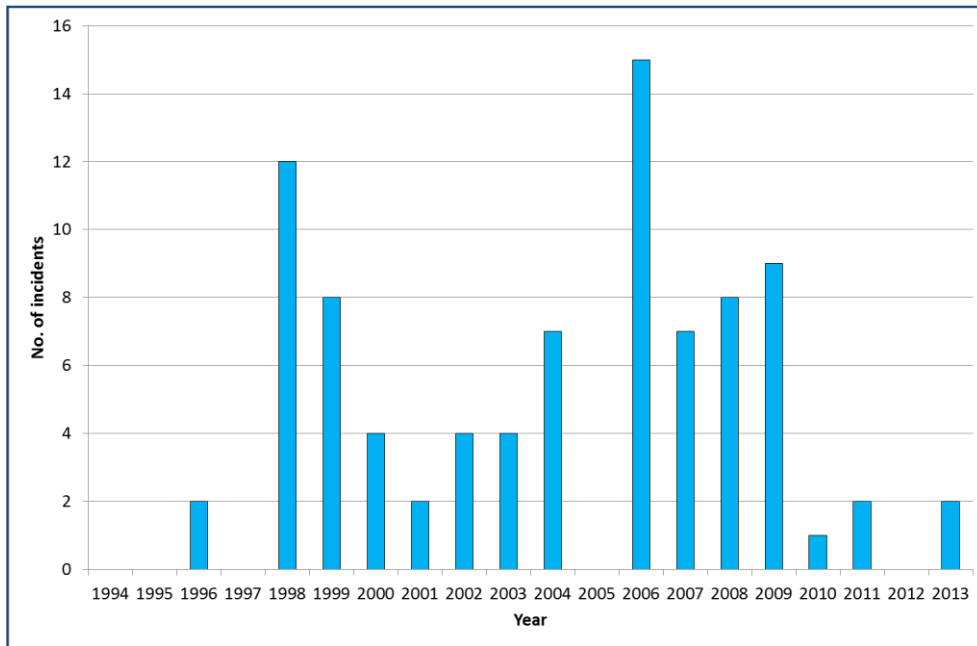
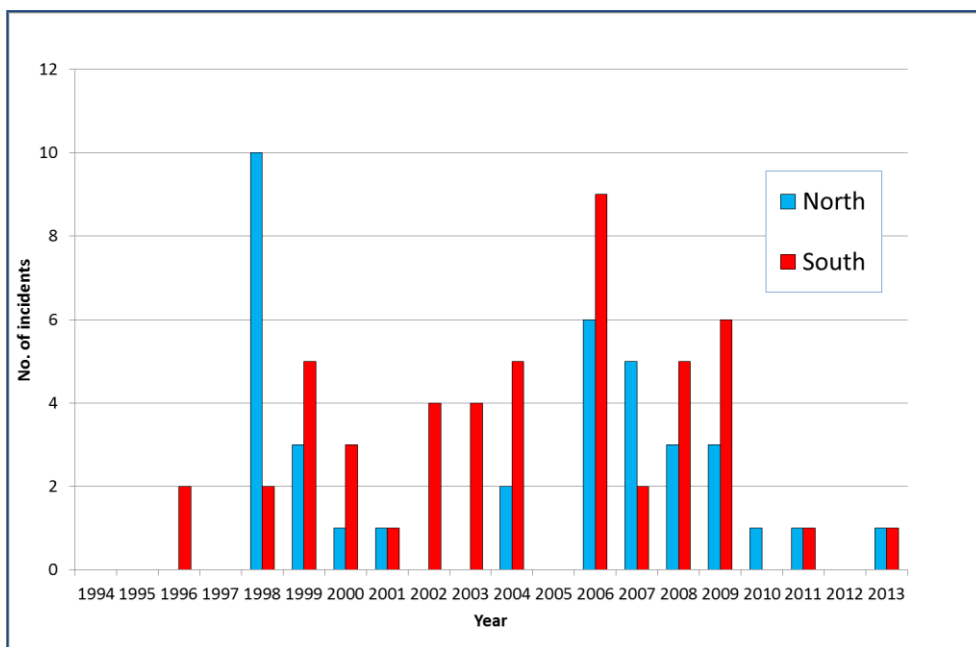


Figure 5.14: *E. albertii* bacteraemia. Number of incidents, by year and region.



5.3.9 *E. albertii* incidents: by month and region

The monthly distribution of *E. albertii* incidents is presented in Figures 5.15 and 5.16. In the north of Scotland, most incidents started in April to July, but in the south incidents tended to start earlier, from February to May.

Figure 5.15: *E. albertii* bacteraemia. Number of incidents, by month.

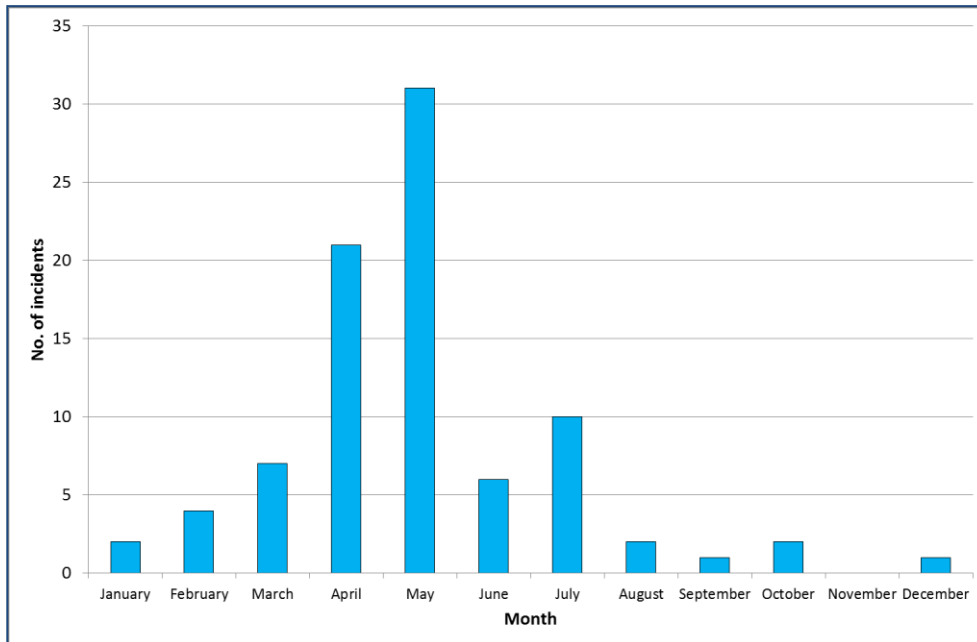
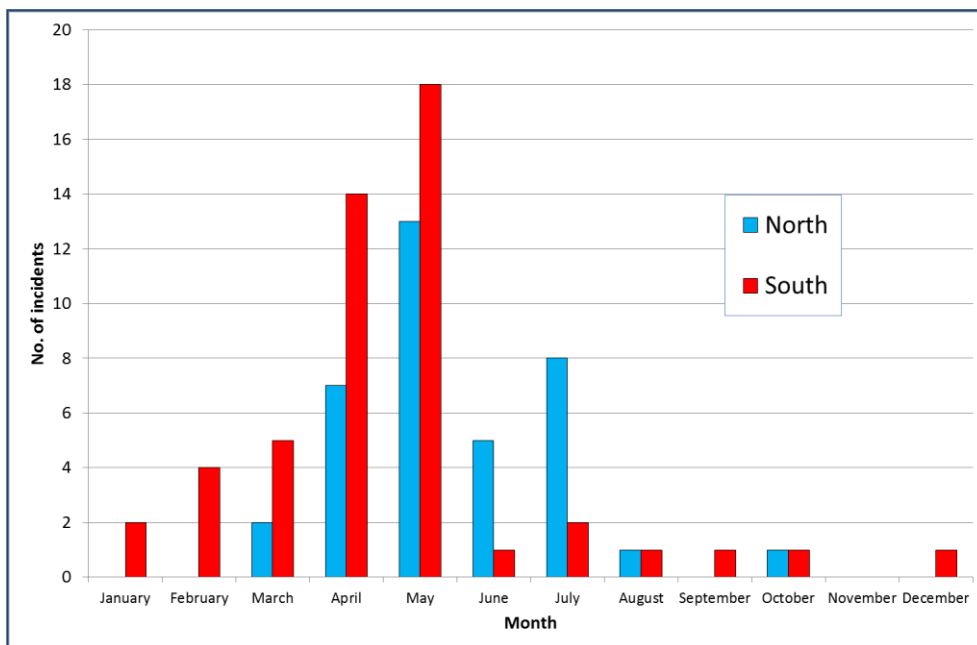


Figure 5.16: *E. albertii* bacteraemia. Number of incidents, by month and region.



5.3.10 Sex and age of birds dying from *E. albertii* bacteraemia: by month and species

For carcasses in which the sex was confidently identified, significantly more cases of deaths from *E. albertii* bacteraemia were diagnosed in male birds (90 adult males, 28 adult females, $p < 0.001$, two-tailed binomial test, assuming a theoretical population made up of 50% males and 50% females). The same was true if only siskins were considered (67 adult males, 18 adult females, $p < 0.001$, two-tailed binomial test), but although the same trend was seen in greenfinches it was not statistically significant (16 adult males, 8 adult females, $p = 0.142$, two-tailed binomial test). The monthly distributions of cases of *E. albertii* bacteraemia in adult male, adult female and immature siskins and greenfinches are shown in Figures 5.17 and 5.18. Most of the deaths in female siskins occurred in April to June, but multiple deaths in males were recorded from February to July. The majority of deaths in female greenfinches occurred in April and May, with deaths in males mostly from March to May. All the deaths in immature siskins occurred in the north region, mostly in June and July. Unlike siskins, no deaths in immature greenfinches were recorded.

Figure 5.17: *E. albertii* bacteraemia in siskins. Number of carcasses of males, females and immatures, by month.

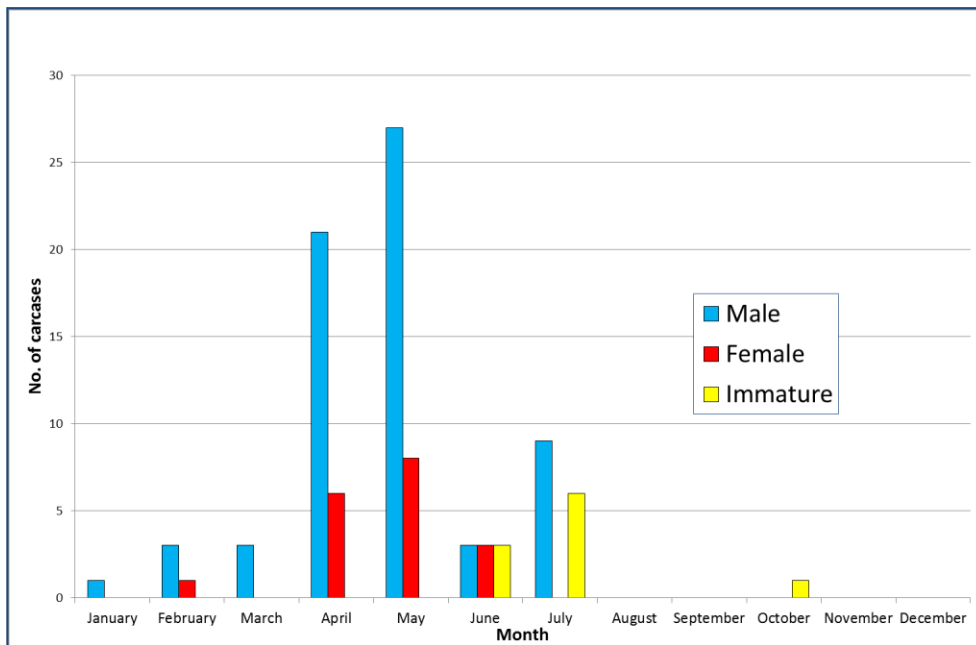
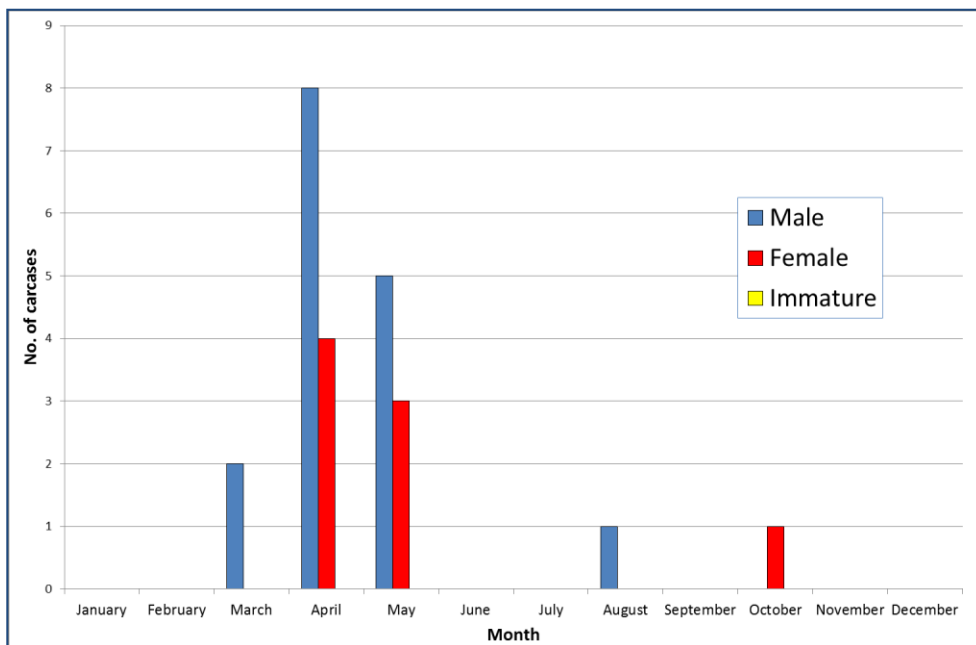


Figure 5.18: *E. albertii* bacteraemia in greenfinches. Number of carcasses of males, females and immatures, by month.



5.3.11 Antimicrobial susceptibility test results

Antimicrobial susceptibility test results for 125 isolates of *E. albertii* from finches are summarised in Appendix IV. Isolates were not tested against all antimicrobials because different panels of antimicrobials were used in disc diffusion tests during the course of the study (see Chapter 2). Most (92.8% [95% CI: 86.4-96.4]) isolates were susceptible to all the antimicrobials tested, but occasional isolates were resistant to ampicillin, amoxicillin, amoxicillin plus clavulanic acid, apramycin, enrofloxacin, neomycin, sulphisoxazole or tetracyclines. Only one isolate was resistant to more than one different group of antimicrobial (enrofloxacin and ampicillin/amoxicillin).

5.3.12 Sorbitol-fermenting strains of *E. albertii*

The preceding results refer to non-sorbitol-fermenting (NSF) strains of *E. albertii* with the API-20E profile of 4144102 or 5144102. In addition, sorbitol-fermenting strains of *E. albertii* with the API-20E profile of 4144502 or 5144502 were recovered from the liver and/or intestine of 3/717 finches (0.4% [95% CI: 0.1-1.3]) and 35/948 non-finches (3.7% [95% CI: 2.6-5.1]). The difference in isolation rates between non-finches and finches was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test). The organism was considered to be an incidental finding in all the birds, and appeared to be commoner in immature birds. Further details of the birds from which *E. albertii* with the API-20E profile of 4144502 or 5144502 was recovered are shown in Table 5.3.

Table 5.3: Further details of birds from which *E. albertii* with the API-20E profile of 4144502 or 5144502 was recovered.

Species	Number	Further details
Blackbird	4	Aspergillosis (immature); trauma (adult); trauma (immature); “orphaned” (immature)
Carrion crow	1	Malnourished fledgling (immature)
Chaffinch	1	Salmonellosis (adult)
Greenfinch	2	Trichomonosis (adult); trauma (adult)
Guillemot	3	Three immature birds in care for 3-4 weeks after seabird “wreck”. All with suspected or confirmed aspergillosis.
Herring gull	1	Suspected botulism (adult)
Immature gull (not further identified)	3	Two immature birds with “swollen head syndrome”; one immature bird with aspergillosis.
Jackdaw	3	Malnourished fledgling (immature); trauma and aspergillosis (immature); trauma (adult)
Rook	1	Abscess in front of eye (adult)
House sparrow	1	Trauma and coccidiosis (immature)
Starling	17	13 birds with fledgling encephalitis (immature); mycotic encephalitis (immature); trauma (immature); adverse environment (immature); “orphaned” (immature)
Coal tit	1	Trauma (adult)

5.4 Discussion

5.4.1 General comments

An overview of the findings for *E. albertii* in finches and other birds is presented here. Further comparisons with salmonellosis and trichomonosis are discussed in Chapter 7, highlighting differences in the patterns seen for these three diseases.

5.4.2 Association of pathogenic *E. albertii* with finches

During the 20 years of this study, the identity and significance of what were initially classified as NLF *E. coli* have become more apparent, with confirmation that the organism is *E. albertii* (Oaks et al., 2010). Isolates of potentially pathogenic *E. albertii* with the API-20E profile 4144102 or 5144102 (NSF strains) were much more likely to be recovered from finches than non-finches, and finch isolates were much more likely to give a positive slide agglutination reaction using O86:K61 antigen than isolates from non-finches. Similarly, deaths from *E. albertii* bacteraemia were only recorded in birds of the finch group. These findings give a very strong indication that this organism is primarily a pathogen of finches, but is not significant in other groups of birds examined. Siskins (52.4% of diagnosable submissions [95% CI: 45.4-59.4]) and greenfinches (7.5% of diagnosable submissions [95% CI: 5.3-10.5]) were the finch species most frequently affected, suggesting that even within the finch group there is varying susceptibility to this organism.

Isolates of *E. albertii* with the API-20E profile of 4144502 or 5144502 (sorbitol-fermenting strains) were more commonly recovered from non-finch species, especially immature birds, and were considered to be incidental findings in birds dying from other causes. They have also been isolated from pooled faecal samples collected from three feeding stations; 8/291 samples (2.7% [95% CI: 1.3-5.5]) from one feeding station, 3/65 (4.6% [95% CI: 1.2-13.8]) from a different site, and 2/33 (6.1% [95% CI: 1.1-21.6]) from a third location (T. Pennycott, unpublished findings). This suggests that sorbitol-fermenting strains may form part of the normal digestive tract flora of some wild birds.

It therefore appears that pathogenicity varies between different strains of *E. albertii*, and further work should be carried out to determine the pathogenicity factors present or absent in different strains. Improved screening protocols for pathogenic strains of *E. albertii* could be developed, using molecular tests or techniques such as immunomagnetic separation and media such as sorbitol MacConkey agar, to aid in the detection of NSF isolates, similar to the methodology used to screen for NSF *E. coli* O157 (Foster et al., 2003; Foster* et al., 2006).

In 151 birds, *E. albertii* bacteraemia was considered to be the cause of death. In addition, the organism was believed to be an incidental finding in a further 23 finches from 20 sites; these were mostly birds dying from trichomonosis, trauma, reproductive tract problems or adverse weather conditions. For six of these birds, deaths from *E. albertii* had been confirmed on that site within the previous three months, but for the remaining 17 birds deaths related to *E. albertii* had either never been confirmed on that site or not within the preceding three months. These findings suggest that this organism may be present in the intestine of some birds without causing problems, and found incidentally in the intestine (or liver if there was agonal bacteraemia or postmortem spread) of birds dying from other causes. This may be especially true for chaffinches, in which the organism was recovered from 19 birds but considered to be incidental in 11 (11/19, 57.9% [95% CI: 34.0-78.9]). This compares with 5/113 (4.4% [95% CI: 1.6-10.5]) in siskins and 4/36 (11.1% [95% CI: 3.6-27.0]) in greenfinches. More targeted screening of finches and other birds would be required to further investigate the role of carrier birds. Further discussions comparing *E. albertii* bacteraemia, salmonellosis and trichomonosis in different species of birds are presented in Chapter 7.

5.4.3 The dominance of *E. albertii* bacteraemia in siskins, and the overall reduction in incidents and carcasses

Deaths from *E. albertii* bacteraemia occurred in fifteen of the twenty years of the study, in both the north and south of Scotland. Deaths in siskins and greenfinches were

recorded in 14 years and 12 years respectively, but chaffinches or goldfinches only in 5 years. In both regions the species of bird most commonly affected was the siskin; 74.3% of all finches dying from the condition in the north were siskins, and 68.8% in the south. Similarly, 75.7% of *E. albertii* bacteraemia incidents in the north involved siskins, as did 72.0% of incidents in the south. Greenfinches were less frequently affected; 20.3% of all finches dying from the condition in the north were greenfinches, as were 22.1% in the south. In contrast with the situation found in siskins, only 29.7% of *E. albertii* bacteraemia incidents in the north involved greenfinches, as did 28.0% of incidents in the south. Chaffinches and goldfinches together made up less than 10% of the carcasses. However, these overall figures mask a change in disease patterns observed during the course of the study, with reductions after 2009 in the number of carcasses, incidents and percentages of diagnosable submissions from siskins and greenfinches in which *E. albertii* bacteraemia was diagnosed. In the latter half of the study, the proportion of cases involving greenfinches also fell significantly. Possible reasons for the reduction in deaths from *E. albertii*, especially in greenfinches, are discussed more fully in Chapter 7, but one obvious factor was the reduction in the greenfinch population caused by trichomonosis (Chapter 6).

5.4.4 Seasonal pattern of *E. albertii* bacteraemia

The seasonal patterns for salmonellosis were described in Chapter 4, with many cases seen between January and March. A different pattern was seen for *E. albertii* bacteraemia, with 65% of confirmed deaths occurring in April to May. Variation between region and species was also noted; most deaths in siskins in the north of Scotland were recorded in April to July, peaking in May and July, but in the south cases tended to be seen earlier, with a peak in April/May and fewer cases in June and July. Deaths in greenfinches also occurred earlier in the south than the north, with most cases diagnosed in April to May in the north compared with March to May in the south.

Possible reasons for the differences in seasonal patterns observed in salmonellosis and *E. albertii* bacteraemia are discussed more fully in Chapter 7, where patterns for the same

species of bird in the same region but with different diseases will be compared. These seasonal differences for birds of the same species and in the same region suggest that a large factor is the epidemiology and pathogenesis of the diseases, rather than factors such as the number of birds visiting gardens in different months of the year.

5.4.5 Sex and age of birds with *E. albertii* bacteraemia

Significantly more deaths from *E. albertii* bacteraemia were diagnosed in adult male siskins than adult females, and the same trend was seen in greenfinches. There was also a seasonal variation in siskins, with males dying between February and July but female deaths concentrated in April to June. Deaths from *E. albertii* were diagnosed in immature siskins but not in immature greenfinches. As indicated in Chapter 4, while it is tempting to speculate that these differences simply reflect greater usage of gardens by male and immature siskins, comparison with patterns seen in other diseases (Chapter 7) indicate that disease epidemiology and pathogenesis play key roles.

5.4.6. Potential zoonotic implications and antimicrobial susceptibility test results

In recent years there has been increasing awareness of the pathogenicity of some strains of *E. albertii* in humans (see 5.2). Although wild bird disease surveillance has highlighted the significance of the organism in certain species of wild birds, more detailed genetic testing of the isolates is required to establish whether wild bird strains of *E. albertii* can be recovered from humans, especially children, with gastro-enteritis. If such a link is established, the results of this study further emphasise the importance of personal hygiene when handling wild birds, their carcasses, bird feeders and the areas around bird feeders that could be contaminated by faeces.

Most isolates of *E. albertii* from finches were susceptible to all the antimicrobials tested, but occasional resistance was noted. Only one isolate was resistant to more than one different group of antimicrobial, and these results support the conclusion that *E. albertii* isolated from finches is a “wild bird” strain rather than a spill-over from humans or livestock, in which prior exposure to antimicrobials would be more likely.

Chapter 6

Trichomonosis in UK finches, sparrows, buntings, dunnocks and tits

6.1 Introduction

Common conditions of UK finches, sparrows, buntings, dunnocks and tits, including salmonellosis and *E. albertii* bacteraemia, are reviewed and discussed in Chapters 3, 4 and 5. This chapter covers the other major problem seen in these birds, namely trichomonosis. Further discussion of salmonellosis, *E. albertii* bacteraemia and trichomonosis is presented in Chapter 7. The Latin names of the UK birds discussed in this chapter are listed in Appendix I.

6.2 Review of trichomonosis in UK finches, sparrows, buntings, dunnocks and tits

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

As described in Chapter 4, ulceration and necrosis of the oesophagus and crop of finches is typically associated with salmonellosis. However, between October 2004 and September 2005 there were several cases of necrotic ingluvitis in finches in the UK from which *Salmonella* sp. was not isolated, and histopathology and examination of wet preparations from some cases showed that the lesions were associated with trichomonad protozoa (Cousquer 2005; Holmes and Duff, 2005; Pennycott* et al., 2005c). This was the first report of disease caused by trichomonads in UK finches, although a recognised problem in pigeons, raptors and budgerigars. Postmortem lesions were usually ulceration and necrosis of the oesophagus and crop, with yellow/orange linear necrosis or discrete nodules. In some birds there was diffuse thickening and necrosis of the oesophageal mucosa, extending to the serosa and resulting in cellulitis under the skin of the neck (Pennycott* et al., 2005c). Oropharyngeal lesions were less common but severe necrosis around the glottis was occasionally reported (Cousquer 2005).

Trichomonad protozoa are actively motile in wet preparations prepared from live or recently dead birds, but the organisms become inactive after the death of their host and are therefore difficult to detect on microscopy of tissues from birds that have been found dead and submitted for necropsy. Culture of swabs or diseased tissues in specific trichomonad media followed by microscopy increases the likelihood of detection of trichomonads for up to eight hours and possibly up to 24 hours after death of the host (Erwin et al., 2000), or where the original intensity of infection is low (Bunbury et al., 2005), but many of the birds in the current study had been dead for over 24 hours prior to necropsy. Later in the epidemic a nested PCR for *Trichomonas gallinae* was developed (Robinson et al., 2010) which did not rely on survival of the trichomonads, but this was only available for use in small numbers of samples. The diagnosis of trichomonosis was, therefore, most often presumptive, based on the presence of oesophagitis or ingluvitis in birds from which cultures for *Salmonella* spp. were negative.

Within twelve months it had become apparent that the number of carcasses presenting with this new condition was escalating, greenfinches and chaffinches being the species most often affected (Lawson* et al., 2006a; Simpson and Molenaar, 2006). SACCVS (Anon* 2008a) reported that the disease in Scotland appeared to have spread in a north-easterly direction since it was first diagnosed in southwest Scotland in 2005, and that in addition to chaffinches and greenfinches, disease had been seen in other finch species such as the bullfinch, goldfinch and siskin, and in non-finches including the house sparrow, dunnock and yellowhammer. This report highlighted a seasonal pattern in Scotland, with most diagnoses being made in the months July to December.

Gene amplification and sequencing subsequently confirmed that the causal organism in the UK was *Trichomonas gallinae*, similar to that found in other species including pigeons and raptors (Robinson* et al., 2010). These authors reported that, in the worst affected parts of England, the breeding populations of greenfinches and chaffinches had

fallen by 35% and 21% respectively, and they estimated that trichomonosis had killed over half a million greenfinches in GB between 2006 and 2007.

Finch trichomonosis had previously been seen in other parts of the world. Reisen et al. (2003) mentioned in passing a condition associated with *Trichomonas* sp. that caused ulceration of the oesophagus and crop of house finches (*Carpodacus mexicanus*) caught from the wild in California, USA, in 2000. Anderson et al. (2009) reported the frequent detection of trichomonads in wild birds including house finches admitted to a wildlife hospital in California between 2001 and 2005, and DNA sequencing showed the organism to be *T. gallinae*. Case fatality in infected house finches was 95.5%. From 2007, trichomonosis was also seen in purple finches (*C. purpureus*) and American goldfinches (*Carduelis tristis*) in the Maritime provinces of Canada (Forzan et al., 2010), although the source of infection remained unresolved.

Finch trichomonosis reached Norway, Sweden and Finland in 2008 (Neimanis et al., 2010). Lawson et al. (2011c), noting the eastward spread of the disease in England, postulated that spread into Fennoscandia may have been the result of chaffinches migrating from the UK. This hypothesis was supported by gene sequencing of *T. gallinae* from finches in Fennoscandia, which proved to be identical to *T. gallinae* from UK finches. In addition to spread to Fennoscandia, finch trichomonosis was reported from Germany in 2009 and France in 2010 (Gourlay et al., 2011), and from Slovenia and Austria in 2012 (Zadravec et al., 2012; Ganas et al., 2014).

Since 2011 there have been several papers reporting the results of genetic sequence analysis of *T. gallinae* from finches, non-finch passerines, raptors and pigeons/doves (Lawson et al., 2011a; Chi et al., 2013; Lennon et al., 2013; Ganas et al., 2014). Although more work is required to definitively differentiate strains, the overall results indicate that disease in finches and non-finch passerines in the UK and in different parts of Europe and Fennoscandia has been caused by a single clonal strain of *T. gallinae*. This “finch epidemic strain” was also the commonest strain found in British pigeons,

doves and birds of prey, supporting the hypothesis that the organism originated in pigeons and doves and then spilled over into passerines.

Updating the impact of trichomonosis in finches in GB, Lawson et al. (2012b) estimated that the disease had reduced the breeding population of greenfinches by 35% between 2006 and 2009, and that the size of flocks visiting gardens had fallen by half. Chaffinch populations were not affected to the same extent and showed some evidence of recovery. Substantial declines were noted in south Finland by Lehtikainen et al. (2013), who found that the breeding population of greenfinches fell by 47% between 2009 and 2010, the wintering population fell by 65%, but chaffinches showed only a small decline. What started as a single confirmed case in a chaffinch in Ayrshire in 2005 escalated into a new/emerging disease of epidemic proportions, with a significant detrimental effect on the population of greenfinches in Europe and Fennoscandia.

6.3 Trichomonosis in finches, sparrows, buntings, dunnocks and tits examined at Ayr DSC 1994 to 2013 – results

6.3.1 Trichomonosis in finches, sparrows, buntings, dunnocks and tits - overview

A diagnosis of trichomonosis was based on the presence of oesophagitis or ingluvitis in birds from which cultures for *Salmonella* spp. were negative. In some birds the diagnosis was additionally confirmed either by observing trichomonads on histopathology, on microscopy of wet preparations from the oesophagus, or after incubation of infected material in Bushby's Trichomonas Medium No. 2 (Oxoid) for 5 days at 30°C. A nested PCR for *T. gallinae* as described by Robinson et al. (2010) also confirmed the disease in siskins, redpolls, chaffinches, greenfinches, a dunnock and a reed bunting.

The results for 1994 – 2013 are presented as follows:

- Overall numbers of carcasses with trichomonosis by year and region (6.3.2)
- Trichomonosis (carcasses) by species and year (6.3.3 – 6.3.5)

- Trichomonosis (carcasses) by species, month and region (6.3.6)
- Trichomonosis (incidents) by species, region and year (6.3.7)
- Trichomonosis (incidents) by species, region and month of onset (6.3.8)
- Trichomonosis (incidents) by sub-region and year (6.3.9)
- Further details of incidents of finch trichomonosis in Scotland in the first year of the epidemic (6.3.10)
- Sex and age of birds with trichomonosis (6.3.11)
- Severity of lesions of trichomonosis (6.3.12)
- Concurrent infections with other potentially pathogenic organisms (6.3.13)

6.3.2 Overall numbers of carcasses with trichomonosis

A diagnosis of trichomonosis was made in 383 out of 1278 submitted birds in this category (30.0% [95% CI: 27.5-32.6]): 373 finches and buntings, 5 house sparrows, 3 dunnocks, 1 tree sparrow and 1 great tit. 284 carcasses with presumed/confirmed trichomonosis originated from the south of Scotland (74.2% [95% CI: 69.4-78.4]), 97 (25.3% [95% CI: 21.1-30.0]) from the north, and no precise location was given for two carcasses. The finch most commonly affected was the greenfinch (179 birds) followed by the chaffinch (146 birds). Trichomonosis was also diagnosed in smaller numbers of siskins (19), goldfinches (17), redpolls (4), bullfinches (4), yellowhammers (2), reed buntings (1) and bramblings (1).

The disease was first seen in 2005 in the south of Scotland, peaking in 2006 and then reducing, but with a small increase again in 2013 (Figure 6.1). Cases in the north were first diagnosed in 2006, gradually rose to a slight peak in 2008 and fluctuated since then (Figure 6.1).

Trichomonosis made up an increasing percentage of total diagnosable submissions of finches, sparrows, buntings, dunnocks and tits (carcasses in which both liver and intestine were cultured to exclude salmonellosis) every year from 2005 to 2008, falling slightly but then increasing again in 2012 and 2013 (Figures 6.2 and 6.3). Overall, trichomonosis

was diagnosed in 43.4% (95% CI 39.1-47.9) of diagnosable submissions between 2005 and 2008, and in 51.1% (95% CI 45.5-56.7) of diagnosable submissions between 2009 and 2013. Between 2005 and 2008 the annual percentage of diagnosable submissions in the south was always higher than in the north (Figure 6.4): overall, 50.3% (95% CI 44.9-55.6) in the south compared with 29.3% (95% CI 22.3-37.4) in the north. However, in the period 2009 to 2013, the overall percentages in the south and north were similar: overall 52.7% (95% CI 45.6-59.7) in the south and 47.3% (95% CI 37.9-56.9) in the north. Trichomonosis accounted for over 40% of all diagnosable submissions from the south in eight years, and in six years exceeded 50%. In contrast, trichomonosis in the north was recorded in over 40% of diagnosable submissions in five years, and reached 50% or more of diagnosable submissions in three years (Figure 6.4).

Figure 6.1: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of carcasses, by year and region.

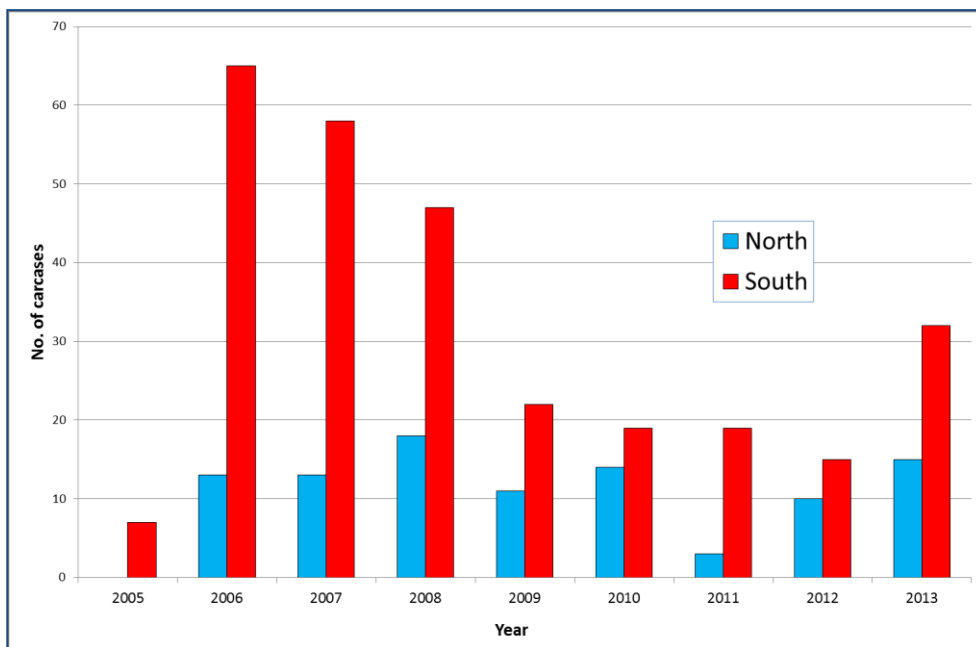


Figure 6.2: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of carcasses with trichomonosis and total number of diagnosable submissions, by year.

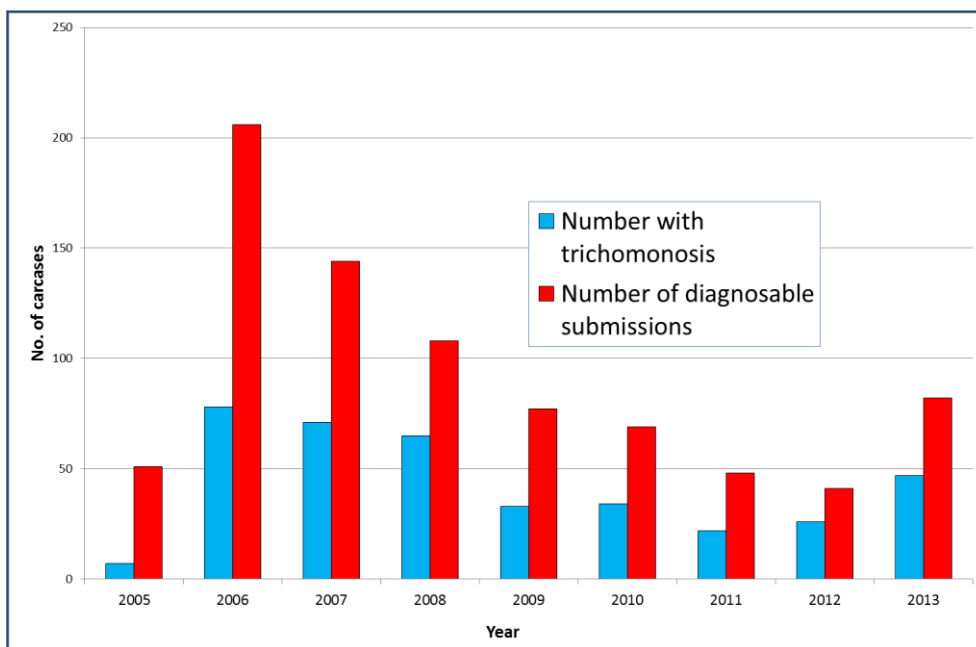


Figure 6.3: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of carcasses as a percentage of diagnosable submissions, by year.

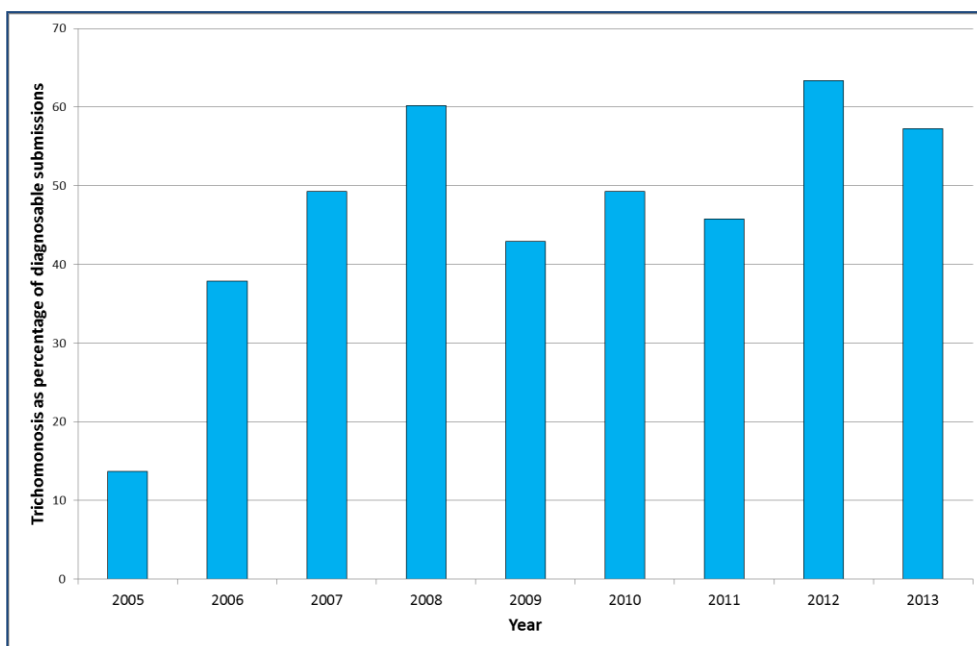
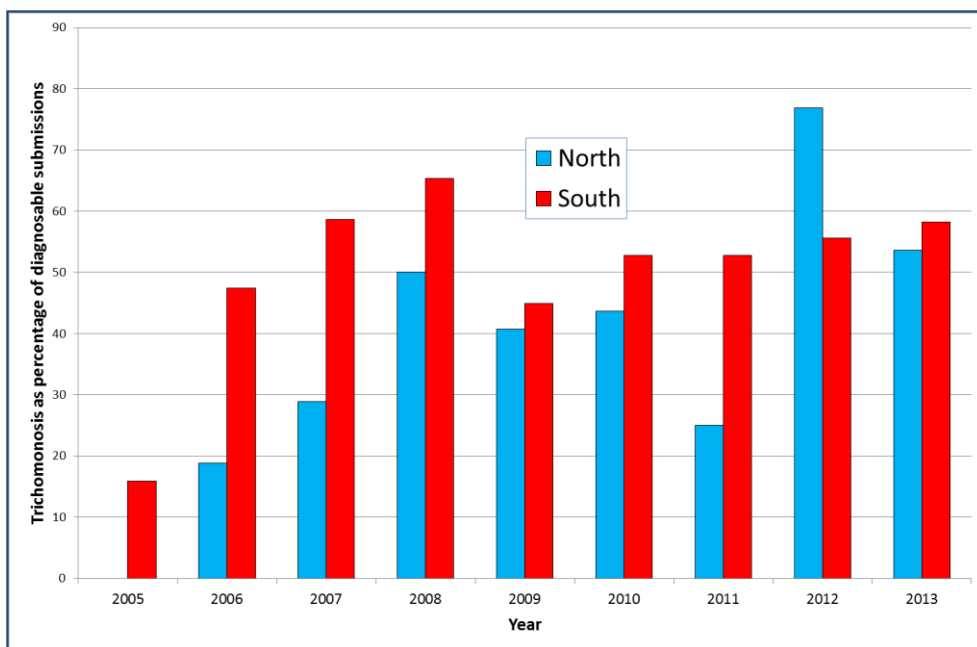


Figure 6.4: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of carcasses as a percentage of diagnosable submissions, by year and region.



6.3.3 Trichomonosis in greenfinches by year

Trichomonosis was recorded in 179 out of 276 greenfinch diagnosable submissions (64.9% [95% CI: 58.9-70.4]). There was a steady increase in the number of greenfinches with trichomonosis between 2005 and 2007, gradually reducing again to 2013 (Figure 6.5). When considered as a percentage of diagnosable submissions, trichomonosis was responsible for over 70% of greenfinch diagnosable submissions in every year from 2007 to 2012, declining in 2013 (Figure 6.6). Figure 6.7 shows the percentage of birds with trichomonosis that were greenfinches: every year from 2007 to 2009, 60% or more of birds with trichomonosis were greenfinches, but thereafter the percentage declined, falling to under 10% in 2013. From 2005 to 2009, 140 out of 254 birds with trichomonosis were greenfinches (55.1% [95% CI: 48.8-61.3]), compared with 39/129 (30.2% [95% CI: 22.6-39.0]) in 2010 to 2013. This difference was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test).

Figure 6.5: Trichomonosis in greenfinches. Number of carcasses with trichomonosis and total number of diagnosable submissions, by year.

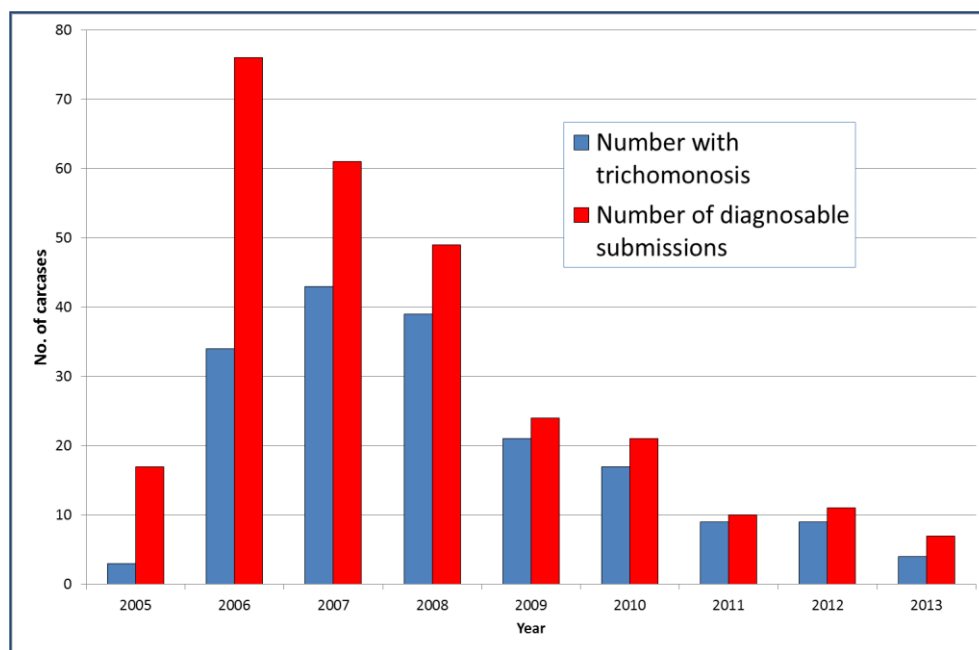


Figure 6.6: Trichomonosis in greenfinches. Number of carcasses as a percentage of diagnosable submissions, by year.

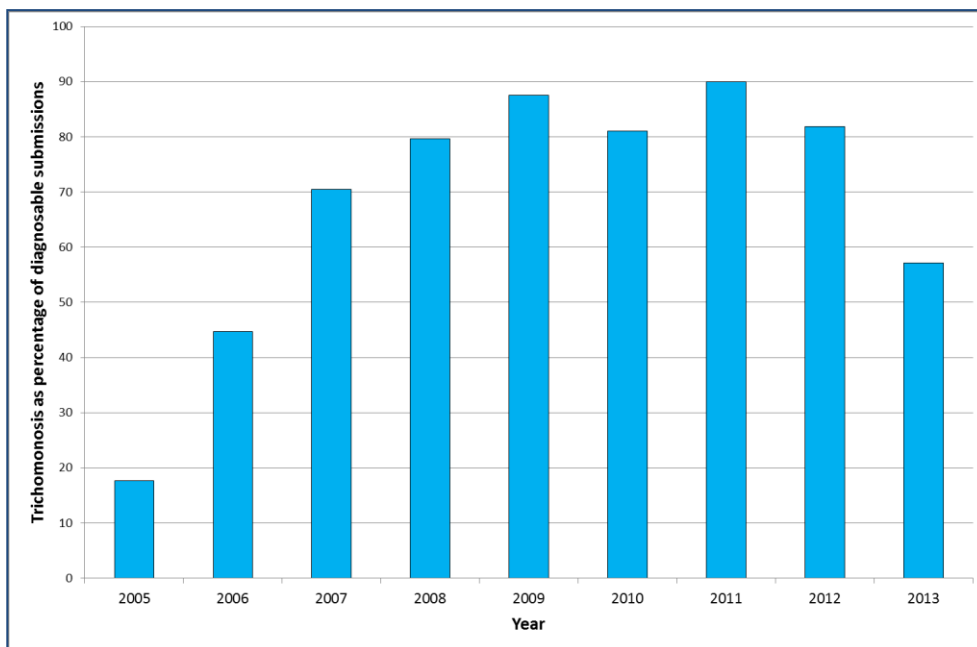
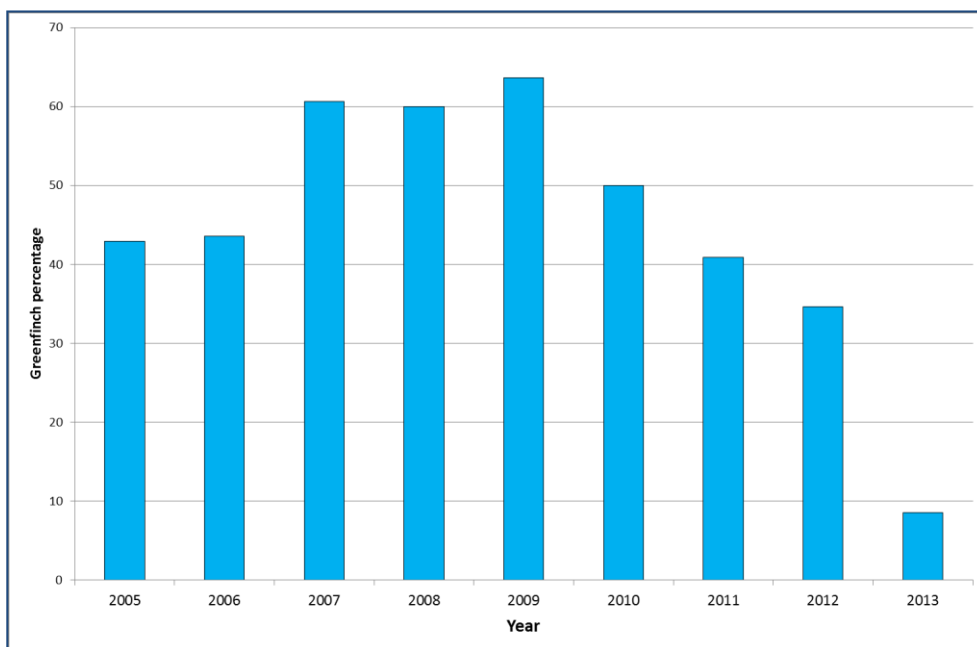


Figure 6.7: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Percentage of carcasses that were greenfinches, by year.



6.3.4 Trichomonosis in chaffinches by year

Trichomonosis was diagnosed in 146 out of 228 chaffinch diagnosable submissions (64.0% [95% CI: 57.4-70.2]). Cases peaked in 2006, reduced in the following years but increased again in 2013 (Figures 6.8 – 6.9). From 2006, the first full year of the finch trichomonosis epidemic, trichomonosis was diagnosed in over 50% of all chaffinch diagnosable submissions every year. In the first two years of the epidemic (2005 and 2006), 51.8% (95% CI: 40.7-62.6) of all cases of trichomonosis occurred in chaffinches, falling over the following four years (29.1% [95% CI: 23.0-35.9]) but rising again (45.3% [95% CI: 35.1-55.8]) in the final three years (Figure 6.10).

Figure 6.8: Trichomonosis in chaffinches. Number of carcasses with trichomonosis and total number of diagnosable submissions, by year.

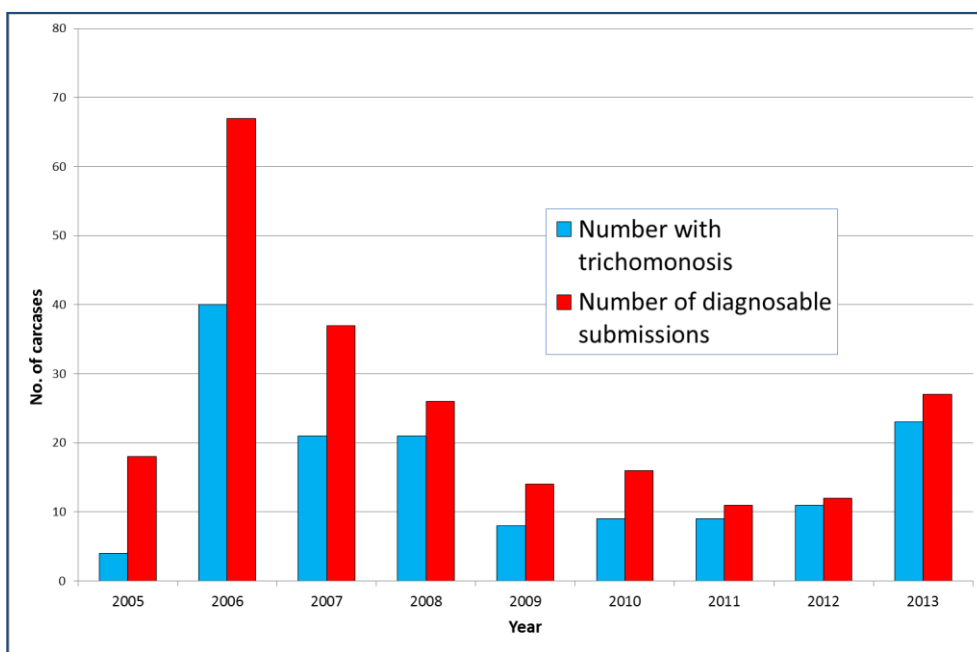


Figure 6.9: Trichomonosis in chaffinches. Number of carcasses as a percentage of diagnosable submissions, by year.

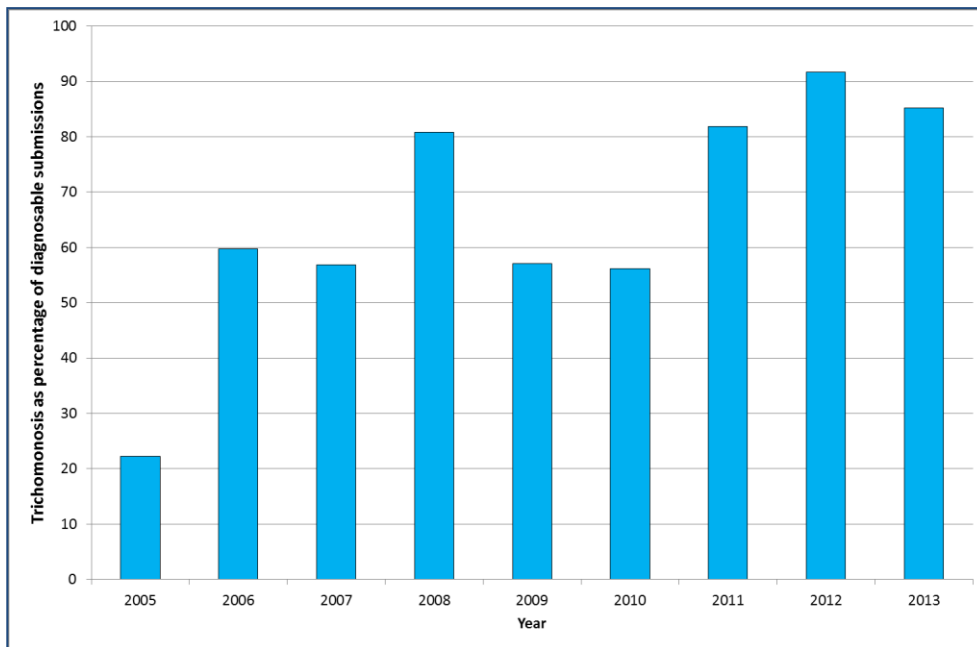
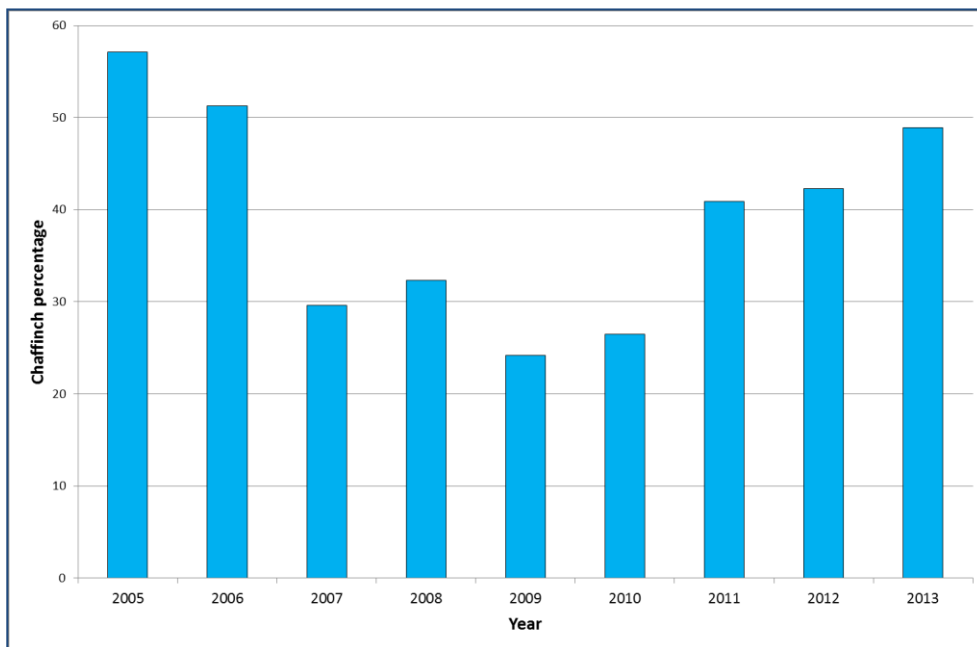


Figure 6.10: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Percentage of carcasses that were chaffinches, by year.



6.3.5 Trichomonosis in birds other than greenfinches and chaffinches by year

For the first five years of the finch trichomonosis epidemic, relatively small numbers of birds other than greenfinches and chaffinches were affected, comprising less than 8% of all cases of trichomonosis in the years 2005 to 2009. From 2010 onwards, non-greenfinches/chaffinches made up a higher percentage of cases, with a clear increase in absolute numbers in 2013 (Figures 6.11 and 6.12). The increase in 2013 largely resulted from greater numbers of siskins and redpolls. From 2005 to 2009, 20 out of 254 birds with trichomonosis were non-greenfinches/chaffinches (7.9% [95% CI: 5.0-12.1]), compared with 38/129 (29.5% [95% CI: 21.9-38.2]) in 2010 to 2013. This difference was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test). Figure 6.13 compares the absolute numbers of greenfinches, chaffinches and "others" (non-greenfinches/chaffinches) over the nine years of the finch trichomonosis epidemic, and reinforces the decline in trichomonosis in greenfinches and rise in "others", the latter substantially exceeding greenfinch numbers in the final year of the study.

Figure 6.11: Trichomonosis in birds other than greenfinches and chaffinches. Number of carcasses with trichomonosis, by year.

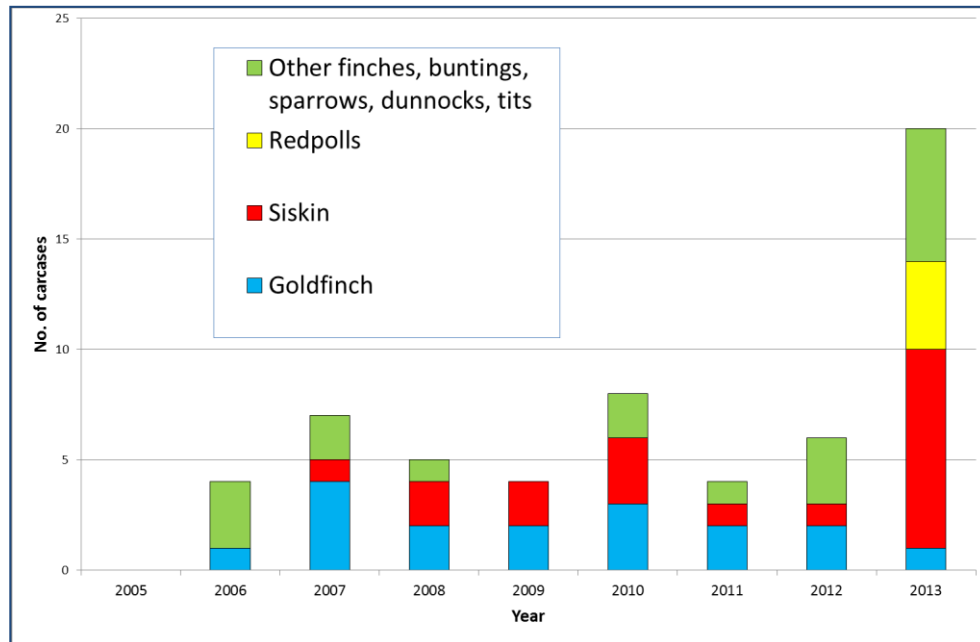


Figure 6.12: Trichomonosis in finches, sparrows, buntings, dunnocks and tits.
Percentage of carcasses that were not greenfinches or chaffinches, by year.

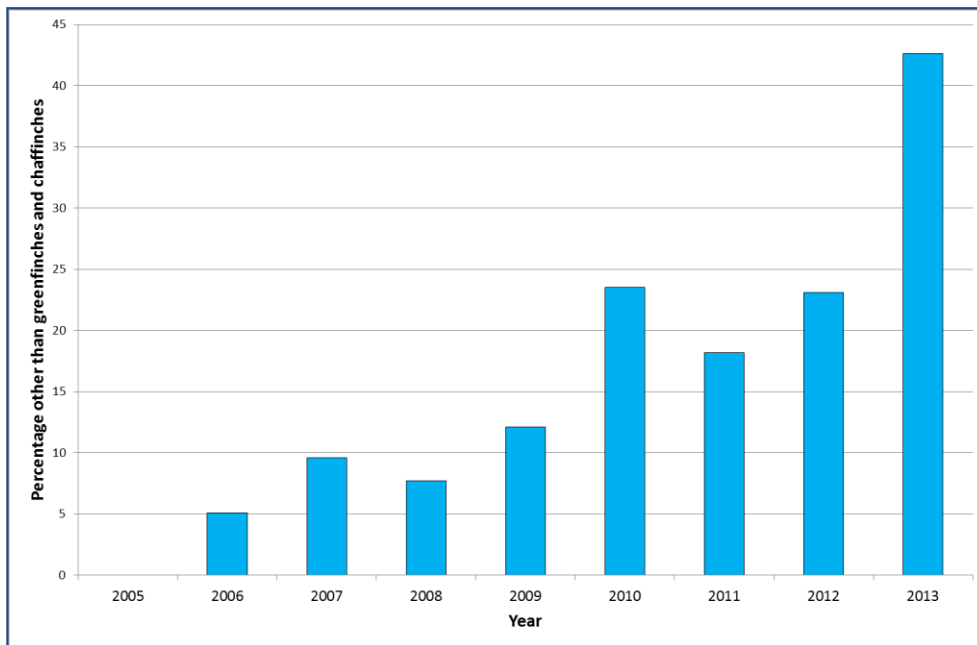
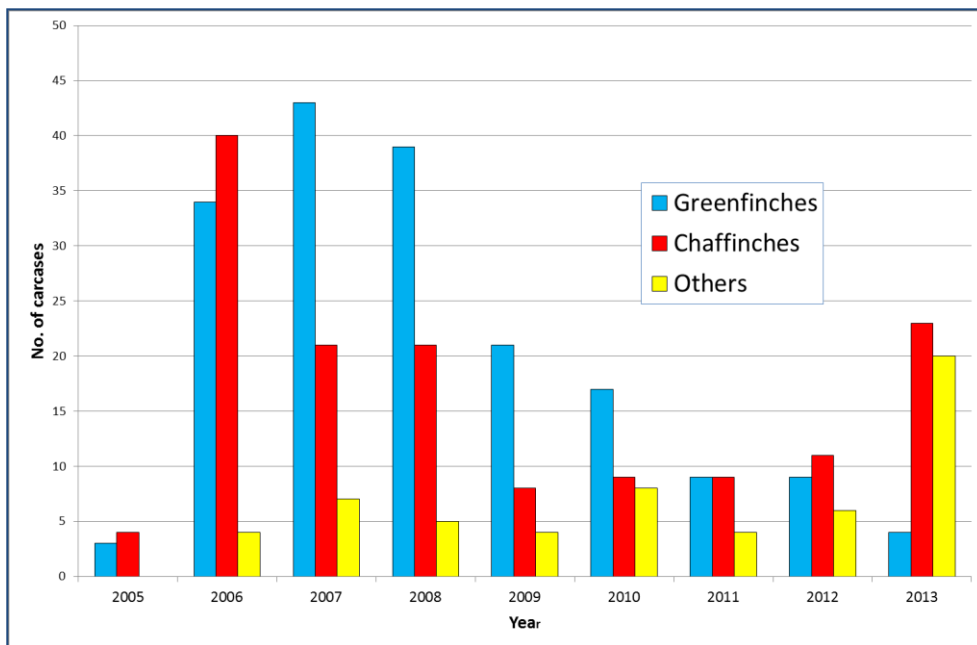


Figure 6.13: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of carcasses of greenfinches, chaffinches and “others”, by year.



6.3.6 Trichomonosis in greenfinches, chaffinches, goldfinches and siskins/redpolls: by month and region

Most (74%) carcasses with trichomonosis originated from the south of Scotland, limiting interpretation of results from the north. In the south, 64.0% (95% CI: 55.4-71.9) of cases in greenfinches occurred in the three months July to September, with a further peak in November (Figure 6.14). For chaffinches in the south, 46.0% (95% CI: 36.1-56.2) occurred in these three months, but with no peak in July and additional peaks in May and November (Figure 6.15). Comparing greenfinch and chaffinch numbers in the south, chaffinch numbers exceeded greenfinch numbers in each month January to May, but the converse was true for June to December (Figure 6.16). This difference between greenfinches and chaffinches was statistically significant ($p < 0.01$, two-tailed Fisher's exact test), and was largely caused by reduced numbers of greenfinches in May to June rather than increased numbers of chaffinches. Smaller numbers of cases of trichomonosis were recorded in the north of Scotland, but as for the south of Scotland cases in chaffinches exceeded greenfinches in each month January to May (Figure 6.17). Chaffinch cases also marginally exceeded greenfinches in August and September in the north. As for the south, the differences between greenfinches and chaffinches in the north were statistically significant ($p < 0.01$, two-tailed Fisher's exact test). Trichomonosis in goldfinches in the south peaked in May, September and November, but numbers were small (Figure 6.18). A peak of cases of trichomonosis in siskins/redpolls in the south was evident, with 76.5% (95% CI: 49.8-92.2) of diagnoses recorded in the two months May and June (Figure 6.19).

Figure 6.14: Trichomonosis in greenfinches. Number of carcasses, by month and region.

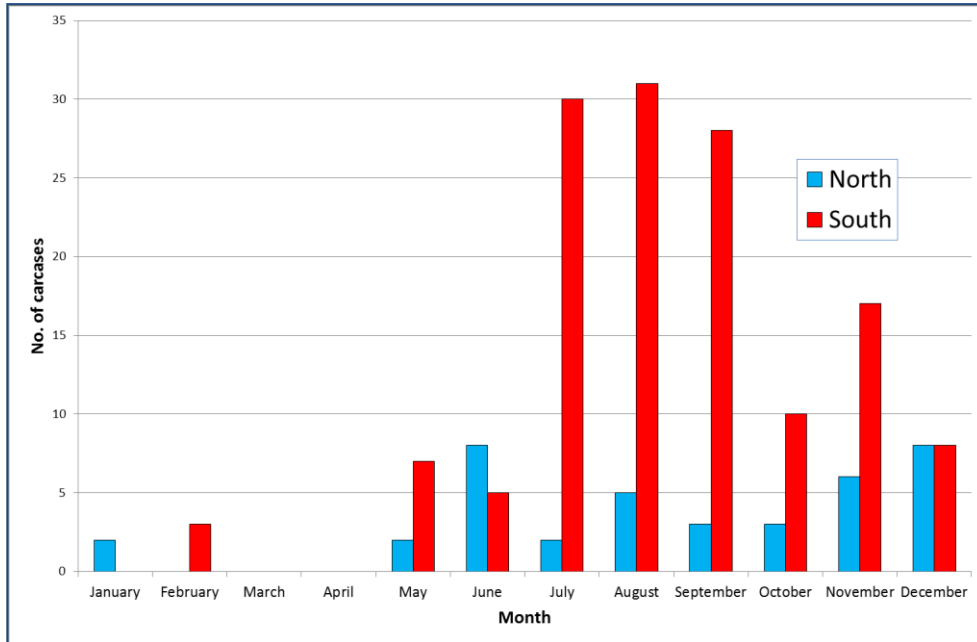


Figure 6.15: Trichomonosis in chaffinches. Number of carcasses, by month and region.

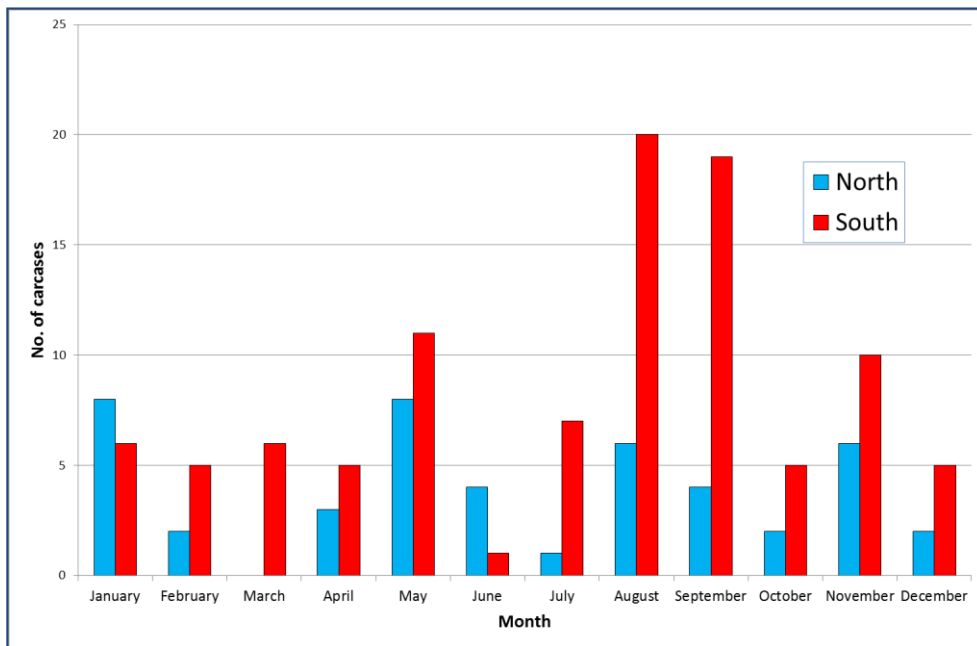


Figure 6.16: Trichomonosis in greenfinches and chaffinches. Number of carcasses in the south of Scotland, by month.

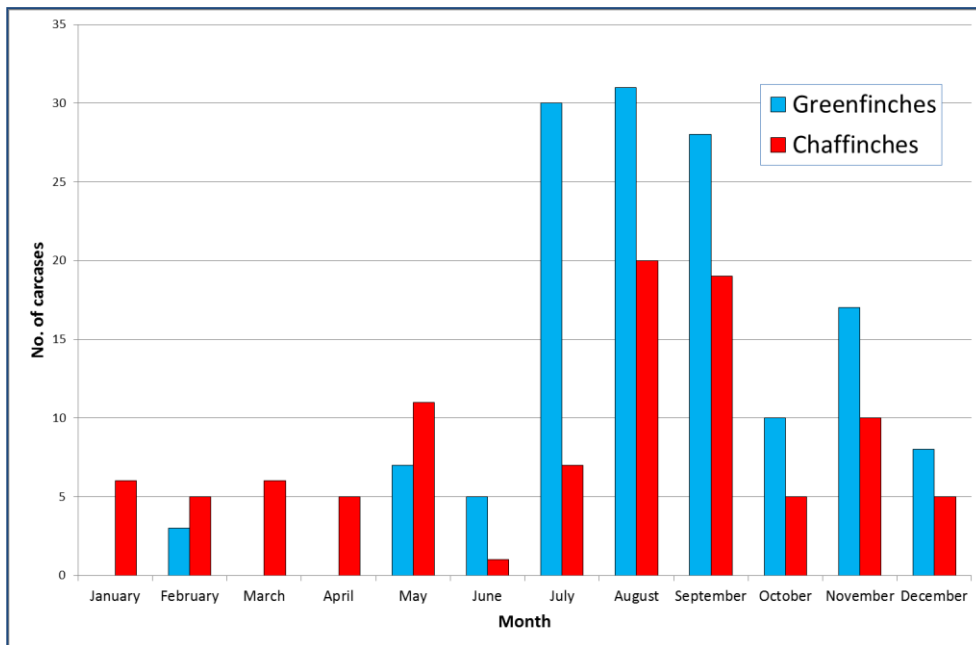


Figure 6.17: Trichomonosis in greenfinches and chaffinches. Number of carcasses in the north of Scotland, by month.

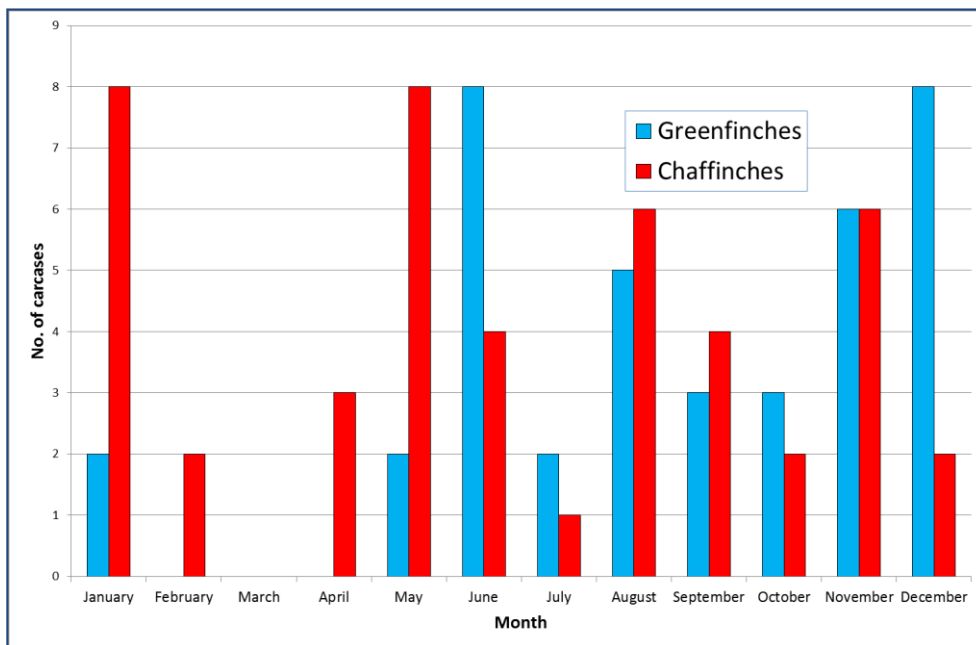


Figure 6.18: Trichomonosis in goldfinches. Number of carcasses, by month and region.

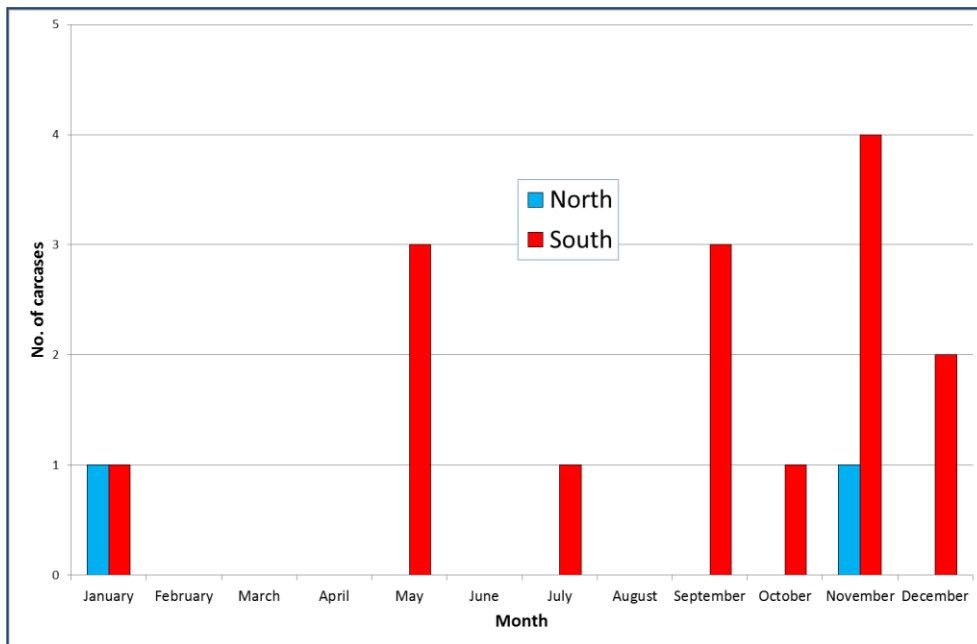
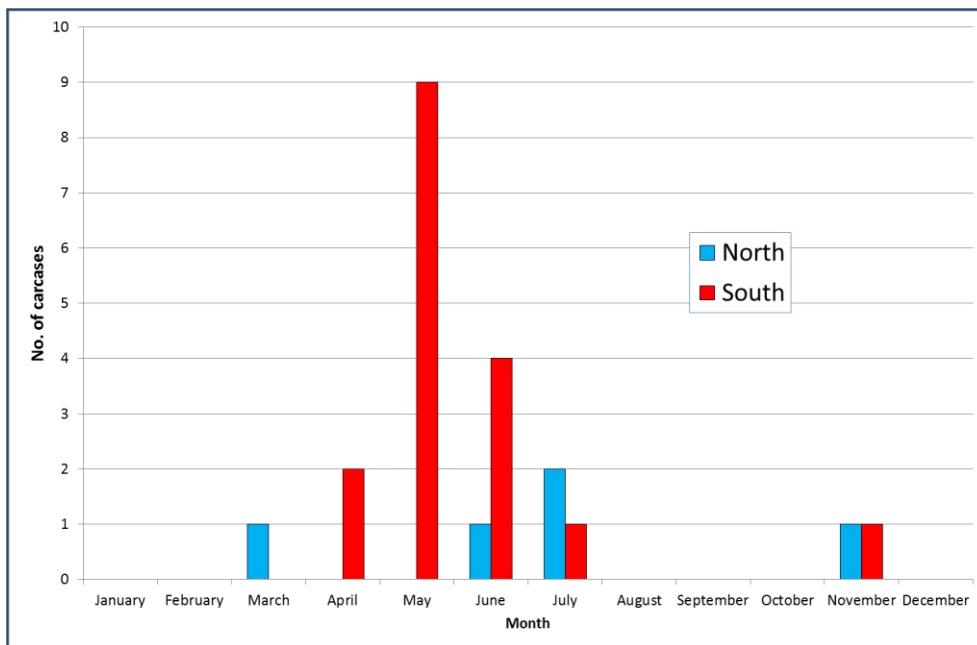


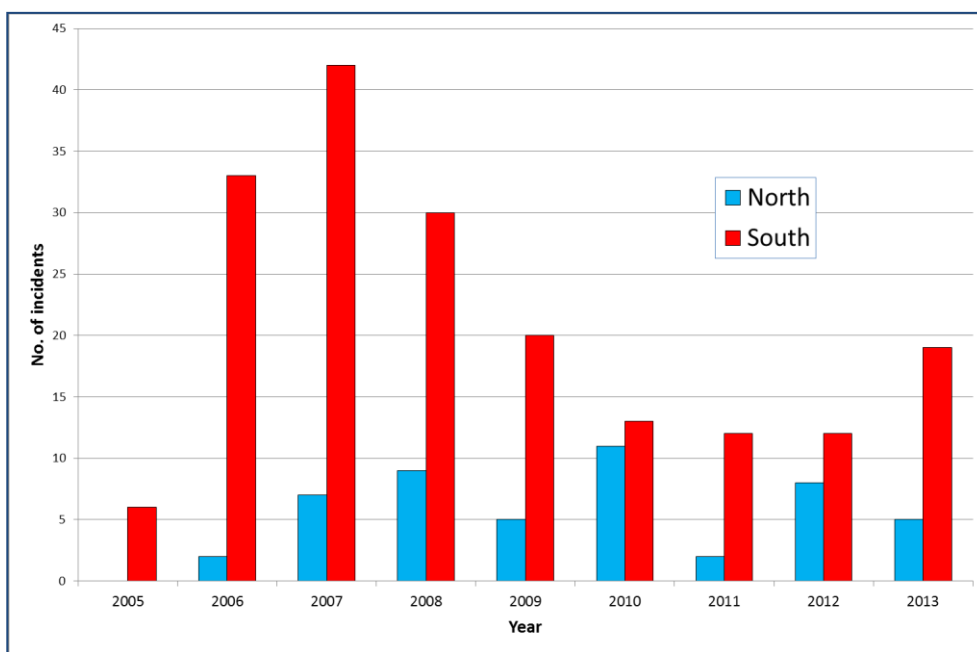
Figure 6.19: Trichomonosis in siskins / redpolls. Number of carcasses, by month and region.



6.3.7 Incidents of trichomonosis in finches, sparrows, buntings, dunnocks and tits: by region and year

A case of trichomonosis was recorded as a new “incident” if there had been at least three months since the same diagnosis had been made in a carcass submitted from that site. Overall, 238 incidents were recorded, of which 187 (78.6% [95% CI: 72.7-83.5]) occurred in the south of Scotland and 49 (20.6% [95% CI: 15.7-26.4]) in the north. In two incidents no precise location was given. These figures are very similar to the number of carcasses with trichomonosis (see 6.3.2) – 74.2% from the south and 25.3% from the north. In the south, the number of incidents rose between 2005 and 2007, declined to 2012 but then increased again in 2013 (Figure 6.20). No trichomonosis incidents were recorded in the north in 2005 and numbers thereafter fluctuated more than in the south (Figure 6.20).

Figure 6.20: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of incidents of trichomonosis, by region and year.



6.3.8 Incidents of trichomonosis in finches, sparrows, buntings, dunnocks and tits: by region and month of onset

When expressed as month of onset of incidents from 2005 to 2013, nearly half (47.1% [95% CI: 39.8-54.5]) of incidents in the south commenced in the third quarter of the year (July to September), and 34.7% (95% CI: 22.1-49.7) of incidents in the north started in the fourth quarter (October to December) (Figure 6.21). However, if further subdivided into three time periods (2005-2007, 2008-2010 and 2011-2013), it can be seen that changes to the temporal patterns have taken place (Figures 6.22 and 6.23). In the south in 2005-2007, most (88.9%) new incidents commenced in the third and fourth quarters of the year. By 2011-2013, only 32.6% started in these months, and 48.8% of new incidents commenced in the second quarter (April to June). Changes over time were also seen in the north: in 2005-2007, 66.7% of new incidents were recorded in the fourth quarter (October to December) and only 11.1% in April to June, but by 2011-2013 the percentage occurring in April to June had increased to 40.0%.

Figure 6.21: Trichomonosis in finches, sparrows, buntings, dunnocks and tits. Number of incidents of trichomonosis, by region and month of onset. 2005-2013.

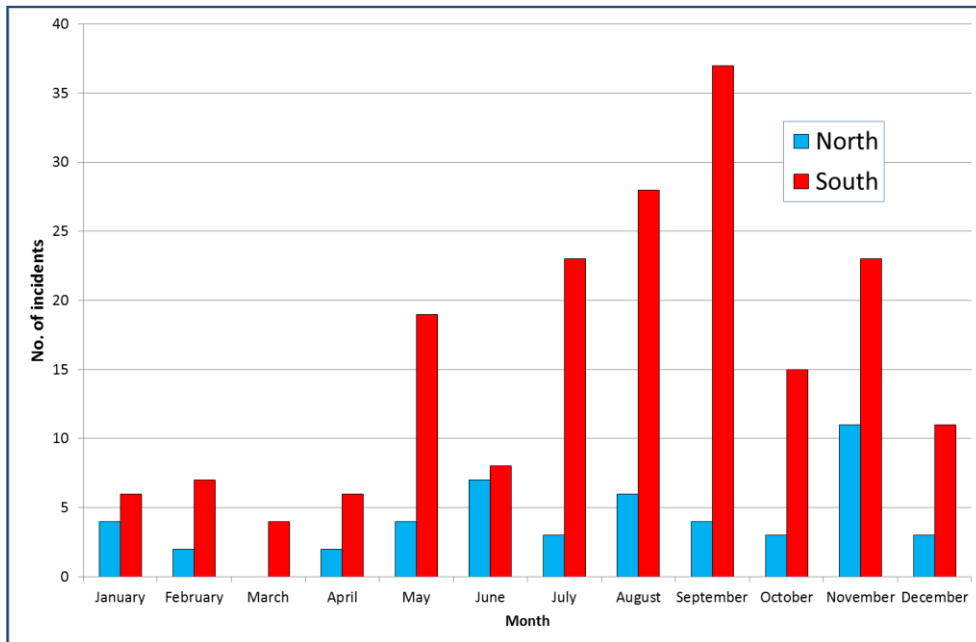


Figure 6.22: Trichomonosis in finches, sparrows, buntings, dunnocks and tits.
Percentage of incidents occurring in different quarters of the year – south of Scotland.

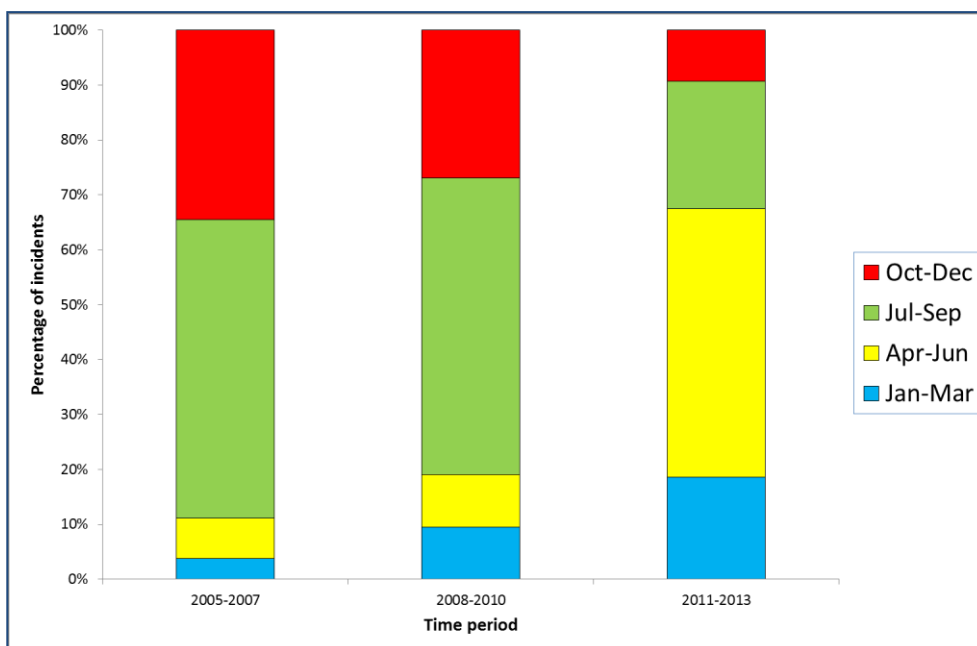
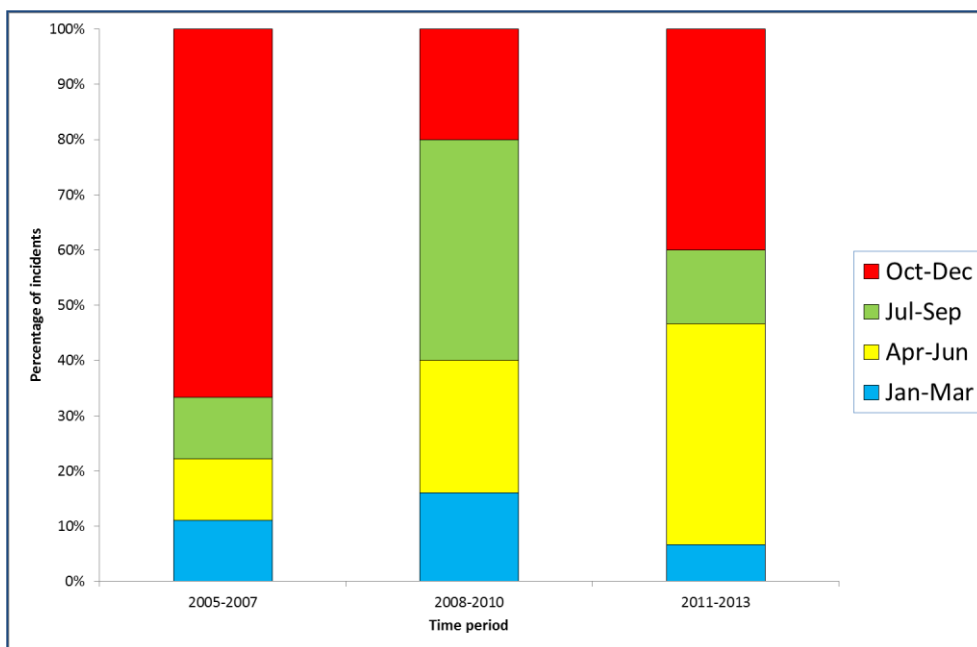


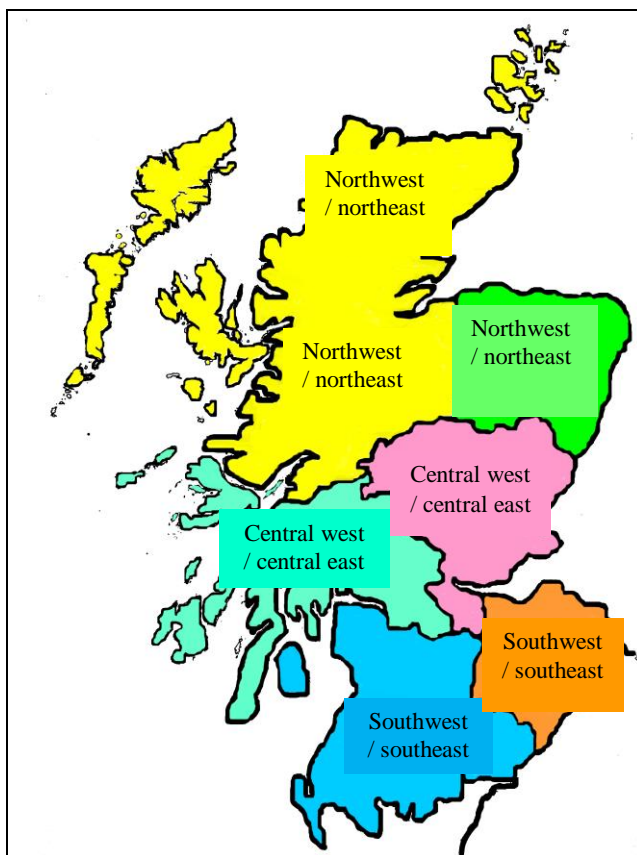
Figure 6.23: Trichomonosis in finches, sparrows, buntings, dunnocks and tits.
Percentage of incidents occurring in different quarters of the year – north of Scotland.



6.3.9 Incidents of trichomonosis in finches, sparrows, buntings, dunnocks and tits: by sub-region and year

To further explore the temporal and geographic spread of trichomonosis within Scotland, the country was divided into three sub-regions: southwest and southeast areas combined, central west and central east areas combined, and northwest and northeast areas combined (Figure 6.24).

Figure 6.24: Division of Scotland into three subregions



The numbers of new incidents recorded in each sub-region in each year are shown in Table 6.1, and the total in each sub-region compared with the number of diagnosable submissions received. In the first year of the epidemic (2005), incidents were restricted to the southwest/southeast of Scotland. The following year (2006), incidents still predominated in the southwest/southeast, but a few incidents were also recorded in central Scotland and in the northwest/northeast. The number of incidents in southwest/southeast Scotland stabilised in 2007, but with increased numbers in the

central west/central east and northwest/northeast sub-regions. In 2008, there was a reduction in incidents in the south, although numbers in the central and northern sub-regions remained constant. Thereafter, the number of incidents in the different sub-regions fluctuated between years. The peak numbers of incidents in the southwest/southeast occurred in 2006 and 2007, in central west/central east in 2007 and 2008, and in northwest/northeast Scotland in 2008 and 2010. Overall the greatest number of carcasses and incidents occurred in southwest/southeast Scotland. When expressed as the percentage of diagnosable submissions in which trichomonosis was diagnosed, the greatest percentage occurred in the south, then the central and then the north sub-regions, but the differences were small.

Table 6.1: Trichomonosis incidents in finches, sparrows, buntings, dunnocks and tits in different sub-regions of Scotland.

	Southwest/ southeast	Central west/ central east	Northwest/ northeast
Incidents in 2005	6	0	0
Incidents in 2006	30	3	2
Incidents in 2007	27	15	7
Incidents in 2008	14	16	9
Incidents in 2009	15	5	5
Incidents in 2010	9	4	11
Incidents in 2011	7	5	2
Incidents in 2012	7	5	8
Incidents in 2013	10	9	5
Total number of trichomonosis incidents	125	62	49
Total number of carcasses with trichomonosis	194	92	97
Number of diagnosable submissions	364	192	269
Percentage (with 95% confidence intervals) of diagnosable submissions with trichomonosis	53.3% (48.0-58.5)	47.9% (40.7-55.2)	36.1% (30.4-42.1)

6.3.10 Further details of incidents of finch trichomonosis in Scotland in the first year of the epidemic (April 2005 to March 2006)

All incidents of trichomonosis in finches in Scotland in the first twelve months of the epidemic (April 2005 to March 2006) occurred within 15 miles of the coast (Table 6.2). The first three incidents occurred in the spring and summer of 2005 near the coast of Ayrshire in southwest Scotland. In the winter of 2005/2006, incidents were seen near the Ayrshire and Solway coasts in southwest Scotland, but also near the Berwickshire coast in southeast Scotland and the Dornoch Firth coast in northeast Scotland.

Table 6.2: Locations of incidents of finch trichomonosis in Scotland in first year of epidemic (April 2005 to March 2006).

Month/year	Species	Method of diagnosis	Location
April 2005	Chaffinch	Trichomonad-like protozoa visible on histopathology of crop	12 miles from Ayrshire coast, southwest Scotland
July 2005	Greenfinch	Gross lesions in crop, absence of <i>Salmonella</i>	5 miles from Ayrshire coast, southwest Scotland
August 2005	Chaffinch	Motile trichomonads detected in wet preparations from crop	15 miles from Ayrshire coast, southwest Scotland
November and December 2005	Chaffinch	Trichomonads cultured in Bushby's Trichomonas medium; nested PCR positive.	8 miles from Solway coast, southwest Scotland
December 2005	Chaffinch	Trichomonads cultured in Bushby's Trichomonas medium.	8 miles from Ayrshire coast, southwest Scotland
December 2005	Greenfinch	Nested PCR positive	15 miles from Berwickshire coast, southeast Scotland
January 2006	Greenfinches x 2	Gross lesions in crop, absence of <i>Salmonella</i>	4 miles from Dornoch Firth coast, northeast Scotland
January and February 2006	Chaffinches x 2	Nested PCR positive.	5 miles from Solway coast, southwest Scotland

6.3.11 Sex and age of birds with trichomonosis

The monthly distribution of adult male, adult female and immature birds is shown in Figure 6.24 (greenfinches) and Figure 6.25 (chaffinches). Of 131 adult greenfinches with trichomonosis, 88 were male (67.2% [95% CI: 58.4-75.0]) and 43 were female (32.8% [95% CI: 25.0-41.6]). Assuming a population based on 50% males and 50% females, the over-representation of male birds was statistically significant ($p=0.008$, two-tailed binomial test). However, if subdivided into quarters of the year, only in the fourth quarter (October to December) was this difference statistically significant ($p<0.001$, two-tailed binomial test). Of adult chaffinches, 83 were male (71.6% [95% CI: 62.3-79.3]) and 33 were female (28.4% [95% CI: 20.6-37.7]). As for greenfinches, the over-representation of male birds was statistically significant ($p=0.001$, two-tailed binomial test). This difference was statistically significant for January to March ($p=0.026$), July to September ($p=0.007$) and October to December ($p=0.003$) but not statistically significant for April to June ($p=0.638$). No difference between males and females was apparent for adult siskins, in which trichomonosis was diagnosed in 6 males and 7 females. In contrast, although the numbers were small, all 9 adult goldfinches with trichomonosis in which the sex was established were male.

Trichomonosis was diagnosed in 29 immature greenfinches, 25 immature chaffinches, 4 immature goldfinches and 4 immature siskins. When further broken down by region, 3 out of 31 (9.7% [95% CI: 2.5-26.9]) categorised greenfinches with trichomonosis in the north were immature, as were 26/128 (20.3% [95% CI: 13.9-28.5]) in the south. Equivalent figures for immature chaffinches in the north were 5 out of 43 categorised chaffinches (11.6% [95% CI: 4.4-29.5]), and 19/97 (19.6% [95% CI: 12.5-29.1]) in the south. Smaller numbers of immature goldfinches and siskins had trichomonosis: the same trend was seen for goldfinches, in which 0/1 (0%) in the north and 4/12 (33.3%) in the south were immature, but the opposite trend was noted in siskins, in which 2/5 (40.0%) were immature in the north but only 1/11 (9.1%) in the south.

Figure 6.25: Trichomonosis in greenfinches. Number of carcasses of males, females and immatures, by month.

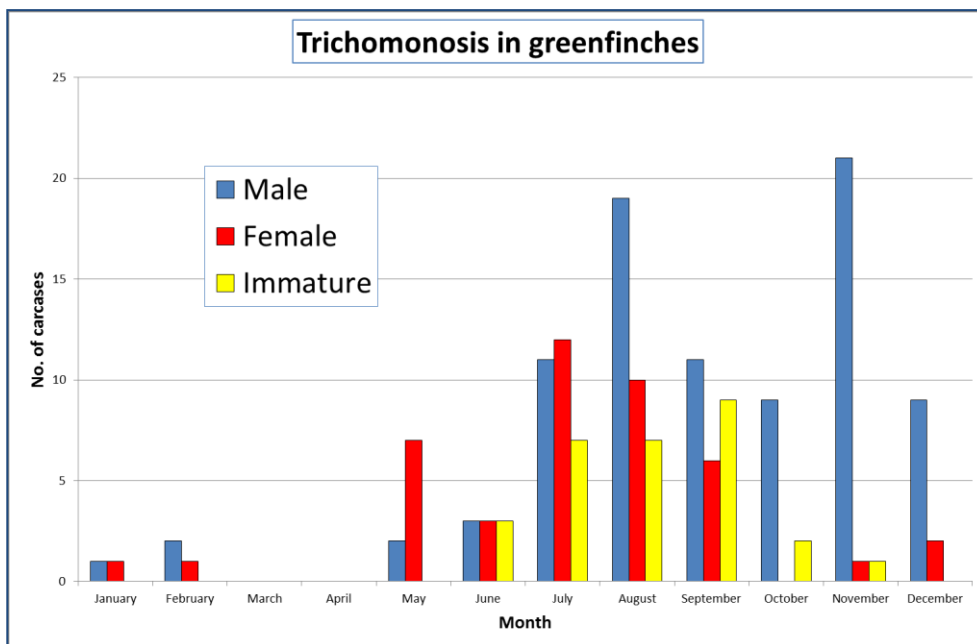
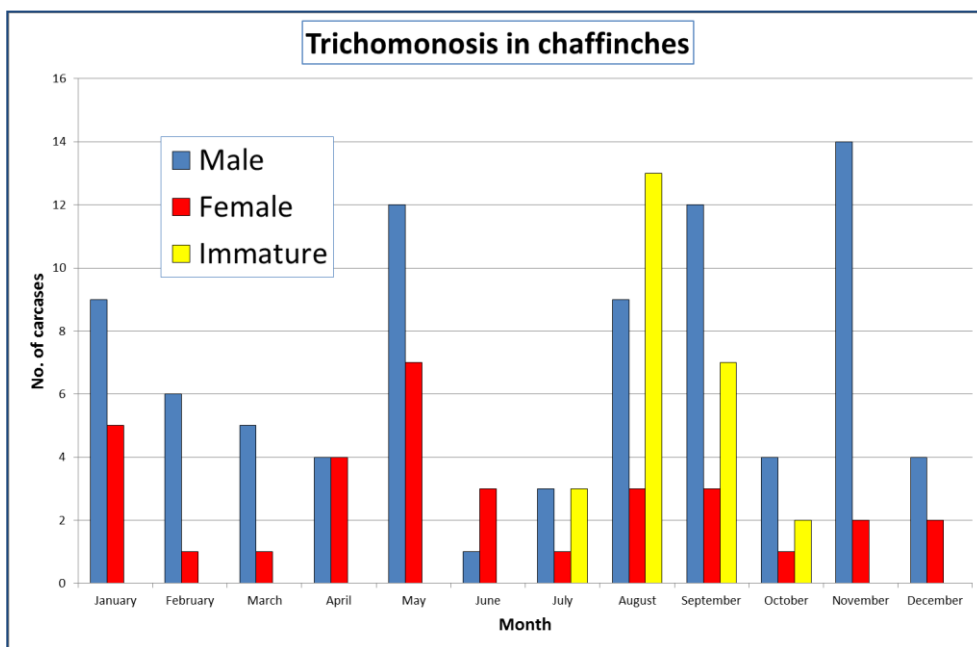


Figure 6.26: Trichomonosis in chaffinches. Number of carcasses of males, females and immatures, by month.



6.3.12 Severity of lesions of trichomonosis

The postmortem lesions in birds with trichomonosis were retrospectively graded based on the recorded descriptions of the lesions. This was carried out in a blind fashion to avoid potential bias. A grade of 1 was given if the oesophagus was described as diffusely reddened or thickened but with no focal or diffuse necrosis; Grade 2 was given if there was focal or diffuse necrosis, usually orange, yellow or white; and Grade 3 was given if there was additional serositis, if the necrosis was so severe that the oesophagus was obstructed or nearly so, or if additional lesions were found in the oropharynx. Images of lesions of trichomonosis in different species of birds can be found in Appendix X ([Images 74-98](#)). Figure 6.27 compares the distribution of lesions in some species of finches and house sparrows. The commonest lesions in greenfinches, chaffinches, goldfinches and siskins were Grade 2, but in redpolls and house sparrows Grade 1 was the commonest lesion. Chaffinches, goldfinches and house sparrows were the species most likely to have Grade 3 lesions. Scores of 2 or 3 were found in a tree sparrow, great tit, bullfinch, buntings and dunnocks with trichomonosis (not included in Figure 6.27). Figure 6.28 demonstrates that when considering all species, over the course of the nine years of the epidemic there was a trend for increasing percentages of Grade 1 (mild) and Grade 3 (severe) lesions, with a corresponding reduction in Grade 2 (moderate) lesions. This was apparent both in greenfinches (Figure 6.29) and chaffinches (Figure 6.30).

Figure 6.27: Trichomonosis: severity of lesions in different species.

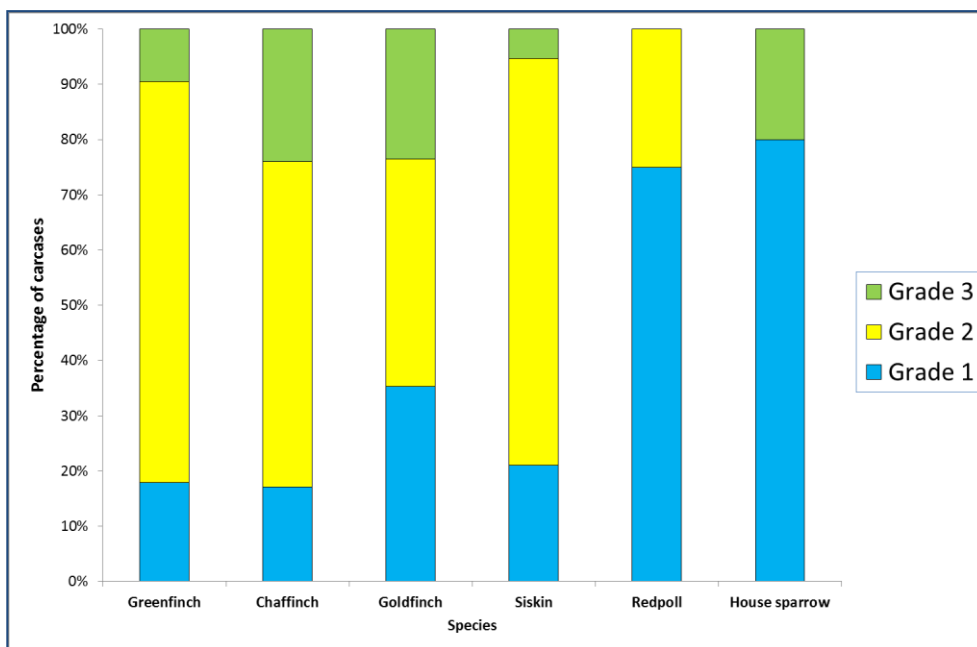


Figure 6.28: Trichomonosis: severity of lesions in all species, in different time periods.

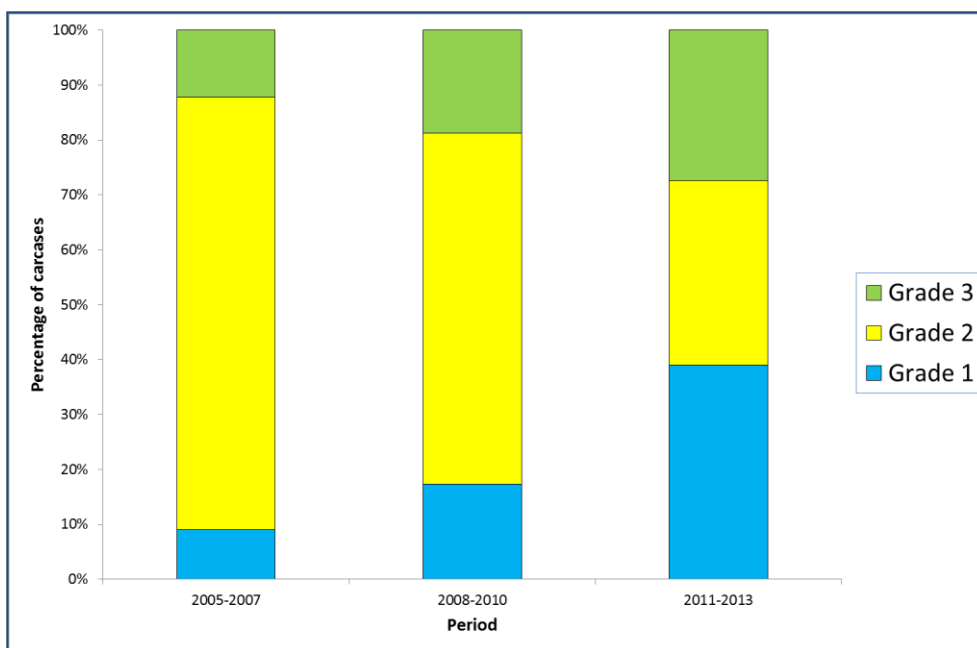


Figure 6.29: Trichomonosis: severity of lesions in greenfinches, in different time periods.

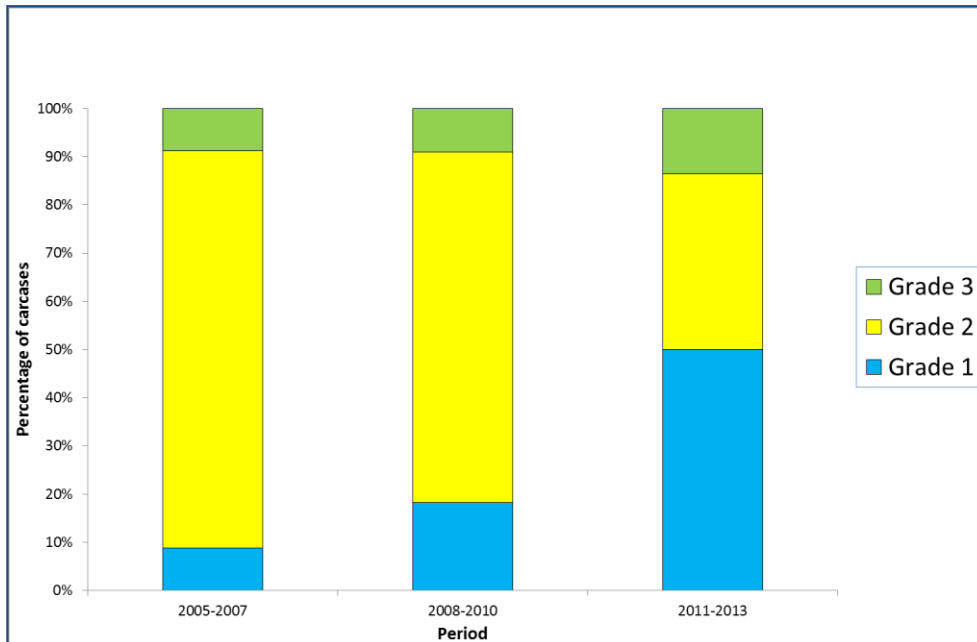
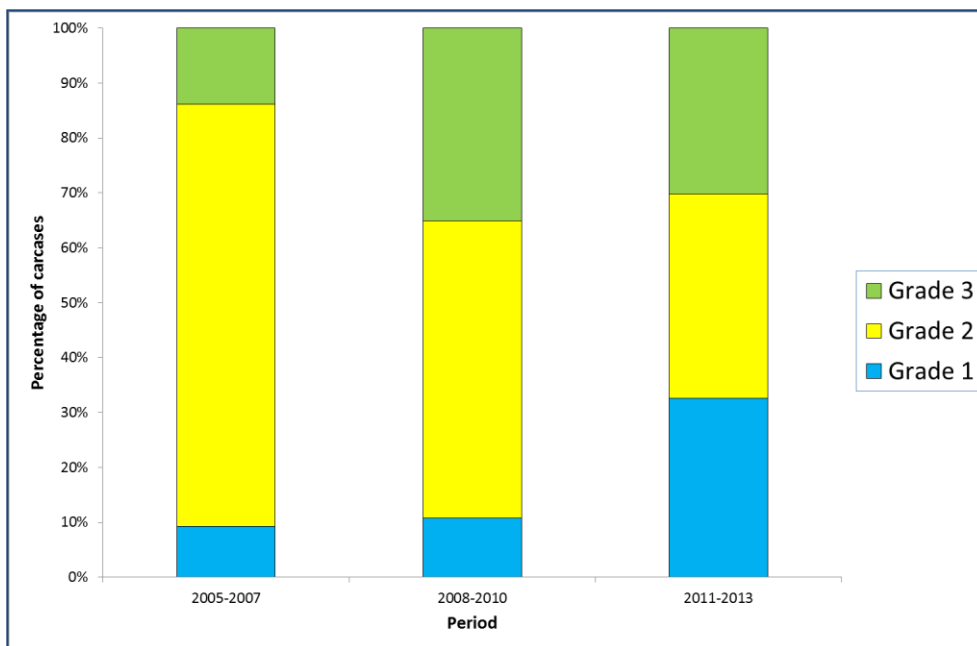


Figure 6.30: Trichomonosis: severity of lesions in chaffinches, in different time periods.



6.3.13 Concurrent infections with other potentially pathogenic organisms

Pathogens other than trichomonads were demonstrated in several birds in which a diagnosis of trichomonosis was made. Potentially pathogenic *E. albertii* was isolated from fourteen finches with lesions of trichomonosis, and *P. multocida* was recovered from two affected birds, suggesting terminal predation by cats. *C. psittaci* was demonstrated by PCR in three finches with trichomonosis, and *Staphylococcus aureus* was isolated from the tissues of three finches and a house sparrow. *Candida albicans* was recovered from a large caseous mass partially obstructing the oropharynx and extending to the side of the face of a chaffinch with trichomonosis (Images 94-96 in Appendix X) – the PCR for *T. gallinae* was also positive. *S. Typhimurium* phage types 193, 40 or 56v were recovered only through selenite from four birds with lesions consistent with either salmonellosis or trichomonosis, and these birds were recorded as cases of trichomonosis based on the diagnostic criteria in Appendix II. PCR testing for *T. gallinae* was carried out on tissues from two of the birds, with positive results, confirming co-infection with these two organisms. On another occasion, three birds with lesions suggestive of trichomonosis or salmonellosis were submitted from one site: *S. Typhimurium* was isolated on direct culture from two birds but not the third, and on the basis of the diagnostic criteria in Appendix II the individual carcasses were diagnosed as salmonellosis (two birds) and trichomonosis (one bird). Subsequent PCR for *T. gallinae* on tissues pooled from the three birds was positive, confirming the presence of both organisms in this incident. It is likely that greater use of the PCR for *T. gallinae* would have revealed more widespread mixed infections.

Two of the three dunnocks with confirmed trichomonosis also had large numbers of schistosome-like eggs in the intestinal mucosa and contents. Similar eggs were found in the intestinal contents of a blackbird with trichomonosis (Chapter 8). It is possible that a pre-existing schistosome-like infection predisposed these “spill-over” species of birds to trichomonosis. Conversely, trichomonosis may have allowed a low level of schistosome-like parasites to substantially increase in numbers. These parasites are discussed more fully in Chapter 8.

6.4 Discussion

6.4.1 General comments

An overview of the findings for trichomonosis is presented in this chapter, and in Chapter 7 the findings will be compared and contrasted with those for salmonellosis and *E. albertii* bacteraemia. Because of the large number of submissions, it was not practical to confirm each case of trichomonosis using additional techniques such as a nested PCR for *T. gallinae*, and delays between death of the birds and examination of the carcasses meant that wet preparations or cultures for *T. gallinae* were often not feasible. Instead, the diagnosis of trichomonosis was usually made on the presence of oesophagitis or ingluvitis in birds from which cultures for *Salmonella* spp. were negative.

6.4.2 Fluctuating numbers of cases of trichomonosis in finches

In the south of Scotland, the overall number of finch carcasses in which trichomonosis was diagnosed peaked in 2006, then fell but rose again in 2013, which was the final year of the study. A similar trend was seen when viewed as incidents rather than carcasses, with a rise in incidents in the south between 2005 and 2007, a decline to 2012 and then a rise again. When considered as the percentage of diagnosable submissions, trichomonosis in the south was responsible for an increasing percentage of diagnosable submissions from 2005 to 2008, fell a little then increased again in 2012 and 2013. These different figures indicate that in the south, where most cases were seen, the disease followed an epidemic curve, before becoming endemic and cyclical.

Trichomonosis in carcasses in the north peaked in 2008, incidents peaked in 2010 and percentage of diagnosable submissions was highest in 2012, but greater fluctuation between years was noted in the north than in the south. These figures suggest that conditions in the north were not as conducive to the maintenance and spread of trichomonosis as in the south, and with fewer cases, no epidemic occurred.

The possible reasons behind the cycles of trichomonosis in finches are explored further in Chapter 7.

6.4.3 Changes in finch species with trichomonosis

Trichomonosis was a major cause of mortality in greenfinches (65% of diagnosable submissions) and chaffinches (64% of diagnosable submissions). The first case in 2005 was in a chaffinch, and in the first two years of the finch trichomonosis epidemic in Scotland, chaffinches were the species most commonly affected. The number of chaffinch carcasses peaked in 2006, fell but then increased again in the final three years of the study. In contrast, the number of greenfinch carcasses with trichomonosis steadily increased between 2005 and 2007, then declined to 2013. Between 2007 and 2010, greenfinches were the species most commonly affected by trichomonosis, but decreasing numbers in greenfinches and increasing numbers in chaffinches resulted in approximately equal numbers of greenfinches and chaffinches by 2011. The decline in greenfinches continued, such that they made up less than 10% of cases in 2013. Initially species other than chaffinches and greenfinches were less affected, but in the final four years of the study other birds (siskins, redpolls, house sparrows) made up an increasing proportion of cases. Possible reasons for the changing pattern of trichomonosis in finches are discussed further in Chapter 7, but partially reflect the deleterious impact of trichomonosis on the greenfinch population.

6.4.4 Spread of trichomonosis into and within Scotland

From a Scottish perspective, incidents of trichomonosis were first recorded in southwest/southeast Scotland in 2005, and central west/central east and northwest/northeast Scotland in 2006. Incidents in the southwest/southeast peaked in 2006 and 2007, in central Scotland in 2007 and 2008, and in the northwest/northeast in 2008 and 2010. The overall direction of spread was from the southwest to the northeast. The greatest number of incidents occurred in the southwest/southeast, then central Scotland, then the northwest/northeast. The same trend was seen when considering the number of carcasses with trichomonosis as a percentage of diagnosable submissions. The

first three confirmed cases in Scotland (April to August 2005) occurred in chaffinches and greenfinches near the southwest coast, and in the first twelve months of the finch trichomonosis epidemic in Scotland, six of the eight positive locations were within 15 miles of the coast in southwest Scotland. Of the remaining two locations, one was near the coast in southeast Scotland and one was near the coast in northeast Scotland. A coastal distribution was also seen in England, where several of the earliest (August to September 2005) cases in greenfinches and chaffinches were recorded near the coast of Cumbria and Lancashire in northwest England (Paul Duff, Paul Holmes and Julian Chantrey, personal communications).

It remains unclear where the finch *T. gallinae* originated from, but the results of genetic sequencing are consistent with a spill-over from pigeons or doves. The appearance of the disease in finches in multiple locations in a short period of time suggests that this spill-over occurred and spread in one or more large groups of birds in close contact with each other, with subsequent dispersal to other parts of the UK. The proximity of many of the first cases to the British coast across the sea from Ireland suggests that this spill-over may have occurred or been amplified in Ireland, with subsequent spread by birds moving back into mainland Britain.

It has been proposed that migrating chaffinches spread trichomonosis to Fennoscandia (Lawson et al., 2011c), and the same species may have been responsible for its introduction into mainland Britain. Although chaffinches that breed in the British Isles are very sedentary, their numbers are almost doubled by migrants from Fennoscandia and north-west Russia (Norman 2002). These birds arrive in mainland Britain from August to December, especially September to November, forming very large flocks that feed and roost together. Some of these birds continue on into Ireland, arriving there in late October/November and leaving again in mid-March to pass through Scotland, England and Wales to return to their breeding grounds further north (Norman 2002). Therefore, the almost simultaneous emergence of finch trichomonosis in multiple sites in mainland Britain in close proximity to Ireland from April 2005 onwards is consistent

with spread by chaffinches migrating from Ireland in the spring of 2005, with subsequent transmission to indigenous chaffinches and greenfinches near the British coast. Migrating chaffinches frequently remain near coasts before moving inland, and although most travel east and return to Fennoscandia through northern Europe, some move north-east and passage through the Scottish Northern Isles (Norman 2002). Infected chaffinches returning to Fennoscandia by this route in the spring of 2005, with subsequent spread of disease to the local population of finches, could explain the later appearance of trichomonosis in greenfinches in northeast Scotland in December 2005.

6.4.5 Seasonal pattern of finch trichomonosis

The seasonal pattern for trichomonosis differed from that for salmonellosis (see Chapter 4) and *E. albertii* bacteraemia (see Chapter 5). In the south, where most trichomonosis occurred, 64% of cases in greenfinches occurred in July to September, plus a November peak. Chaffinch cases in the south were more spread out, 46% occurring in July to September and with other peaks in May and November. Cases in goldfinches in the south were mostly in May, September and November, but in siskins/redpolls they were mostly in May and June. Seasonal differences between species could affect the overall seasonality of the occurrence of disease: if more cases were seen in siskins, redpolls and chaffinches and fewer cases in greenfinches (as occurred in Scotland in recent years), the peak seasonality could change from July-September to April-June. Indeed, such a change in seasonality was observed in Scotland – in the first three years of the finch epidemic in the south of Scotland, 54% of new incidents commenced in July to September and only 6% in April to June, switching to 23% in July to September and 49% in April to June in 2011-2013. The seasonal pattern of trichomonosis is further discussed in Chapter 7, and compared with the seasonal patterns for salmonellosis and *E. albertii* bacteraemia.

6.4.6 Sex and age of birds with trichomonosis

Trichomonosis was diagnosed in more adult male greenfinches and chaffinches than adult females. For greenfinches this was statistically significant for the fourth quarter of

the year (October to December), and for chaffinches this was statistically significant in all quarters except the second (April to June). The same trend was seen in adult goldfinches, but no difference in sex ratio was apparent for trichomonosis in adult siskins. Trichomonosis was also frequently diagnosed in immature birds; between 9% and 40% of cases were in immature birds, depending on species and region. While it is tempting to suggest that the sex ratio and the proportion of immature birds simply reflected the numbers of different categories of birds visiting gardens, it is worth noting that immature birds made up less than 3% of confirmed cases of salmonellosis (Chapter 4). Bird numbers alone cannot account for this difference between trichomonosis and salmonellosis, and further explanations are discussed in Chapter 7.

6.4.7 Severity of lesions in birds with trichomonosis

The lesions found in birds with trichomonosis were graded 1-3 depending on their severity, and over the course of the study there was a tendency for polarisation of the severity of the lesions to mild or severe, with a reducing proportion of lesions graded as moderate. Variation between species in the severity of the lesions was also apparent, with severe lesions more common in chaffinches and goldfinches than in greenfinches, and mild lesions seen more often in redpolls and house sparrows. It appears that different species of bird respond to *T. gallinae* in different ways, as already noted for *S. Typhimurium* (Chapter 4), and that the response can change over time as variations occur in the immune status of the wild bird population and possibly changes in the pathogenicity of the organisms. In a multi-host population as found at feeding stations, differences in host responses could influence disease dynamics, a theme further explored in Chapter 7.

Chapter 7

Finches, sparrows, buntings, dunnocks and tits - some comparisons between salmonellosis, *Escherichia albertii* bacteraemia and trichomonosis

7.1 Introduction

Salmonellosis, *Escherichia albertii* bacteraemia and trichomonosis in finches, sparrows, buntings, dunnocks and tits were discussed in Chapters 4-6. Several questions remain unresolved, and this chapter further explores these issues by comparing the patterns of these three diseases in different species of bird, with reference to:

- transmission dynamics of microparasites at wild bird feeding stations (7.2)
- the contributions of the different diseases to mortality incidents in different species of bird, over time and region (7.3.1-7.3.5)
- the changing balance between salmonellosis, *E. albertii* bacteraemia and trichomonosis (7.3.6)
- the monthly distribution of carcasses with different conditions (7.4)
- differences in ages and sexes of birds affected by different conditions (7.5-7.6)
- the role of supplementary feeding (7.7)
- measures to reduce losses at feeding stations from salmonellosis, *E. albertii* bacteraemia and trichomonosis (7.8)

7.2 Transmission dynamics of microparasites at wild bird feeding stations

Wild bird feeding stations can attract birds of different species, sexes and age groups, some possibly harbouring a range of potential pathogens and all varying in their natural susceptibility or resistance to different diseases. Disease ecology and epidemiology at such locations will therefore be complex. Swinton et al. (2001), discussing the transmission and persistence of microparasites (viruses, bacteria and protozoa) in wildlife, noted that host populations can be divided into those that are susceptible and have never been infected (S), those that are infected and infectious (I), and those that

have experienced infection in the past but have recovered and are no longer infectious (R). Epidemiological models can be constructed using the S:I:R model, based on the proportions of animals present in each compartment at any one time. The basic reproduction ratio or basic reproductive number (R_0) of a condition quantifies the average number of secondary cases that one case generates in a susceptible population. For viral, bacterial or protozoal infections to persist in a population, the average infected host (I) must infect at least one susceptible host (S) before dying or recovering and becoming non-infectious (R) i.e. $R_0 > 1$ (Anderson and May, 1991). The calculation of R_0 may help to predict the outcomes of infection by multi-host pathogens in a multi-species community such as found at bird feeding stations (Swinton et al., 2001; Roche et al., 2012). Factors that affect the final pattern of disease in a multi-host, multi-pathogen environment will include the nature of the pathogen or pathogens, features of the individual bird, and the context (environment and species diversity in the host population) in which contact between the pathogens and individual birds occurs (Roche et al., 2012).

7.2.1 Pathogen factors

Several pathogen factors influence microparasite transmission dynamics and these factors vary between pathogens (Table 7.1). Unfortunately, little work has been done to establish these parameters in wild birds.

Table 7.1: Some pathogen factors that could influence microparasite transmission dynamics

	Pathogen 1	Pathogen 2
Size of infectious dose	High	Low
Main route of transmission	Faecal-oral	Oral-oral
Vertical transmission?	Possibly	No
Main site of persistence	Lower digestive tract. Possibly spleen.	Upper digestive tract
Incubation period prior to clinical signs	Variable	Variable
Magnitude of excreted organisms	Low to high	Low to high
Duration of excretion	Short to long	Short to long
Route of excretion	Faecal	Oral
Persistence in environment	Long	Short
Prevalence in healthy birds	Low	High

It is tempting to assume that *Salmonella* Typhimurium behaves like Pathogen 1 in Table 7.1 and *Trichomonas gallinae* behaves like Pathogen 2, but more detailed research is required to fully establish these parameters for different organisms in wild birds. For *S. Typhimurium* in garden birds, it is unknown if the organism behaves like *S. Typhimurium* and *S. Gallinarum* biovar *Gallinarum* (*S. Gallinarum*) in poultry, which persist in the intestine of a small number of birds and are intermittently excreted for long periods. Alternatively, garden bird salmonellae may behave like *S. Gallinarum* biovar *Pullorum* (*S. Pullorum*) in poultry, persisting in macrophages in the spleen and spreading to the reproductive tract at the onset of sexual maturity (Lister and Barrow, 2008). Hughes et al. (2008) noted that isolates of *S. Typhimurium* from wild birds possessed virulence genes found in both *S. Pullorum* and *S. Gallinarum* and had the ability to invade and persist in avian macrophage-like cell lines, underlining their potential pathogenicity. There is also a suggestion that the virulence of pathogens might increase if, as occurs at feeding stations, the opportunities for transmission are increased (Becker et al., 2015).

7.2.2 Bird species factors

Different species of birds may contribute to the spread of different pathogens at different times of the year depending on their natural biological cycles; examples of some of the differences between greenfinches and chaffinches are shown in Table 7.2. Thus for an organism such as *T. gallinae* that inhabits the oesophagus and crop, there are greater opportunities for transmission among greenfinches than among chaffinches, because male greenfinches (but not chaffinches) feed the females during courtship and when the hen is incubating the eggs. In addition, food is stored in the crop of adult and young greenfinches for longer periods than in the crop of chaffinches, thereby increasing contact between food material and pathogens such as *T. gallinae* and facilitating the spread of pathogens.

Table 7.2: Some bird species factors* that could influence microparasite transmission dynamics

	Greenfinch	Chaffinch
Number of clutches	2-3 (ready availability of seeds for chicks)	1, rarely 2 (limited by caterpillar numbers for chicks)
Territory established by cock	Small, with loose breeding colonies	Large
Courtship feeding	Pair formation and courtship feeding while still in flocks	No courtship feeding prior to nesting
Feeding during incubation of eggs	Hen depends on cock for food during incubation	Hen usually feeds herself
Feeding of chicks	Both sexes	Both sexes but mostly hen
Feeding ecology	Feed from ground and from feeding stations	Mostly feed from ground
Diet of chicks	Mostly seeds. Stored in crop of adult, regurgitated and fed to chick, stored in crop of chick. Large and infrequent meals.	Mostly invertebrates (caterpillars). Small but frequent meals delivered by adults, not stored in crop of chick or adult for a long time.
Migration	Relatively sedentary	Large numbers migrate to UK in winter from Fennoscandia and northern Europe
Flock feeding	All year round	Mostly out-with the breeding season

*Newton 1967, 1972

7.2.3 Individual bird factors

Additional factors acting at the level of the individual bird include:

- Nutritional status. Improved nutrition through supplementary feeding could improve the bird's immune system, reducing R_0 . Conversely, if the food provided was of poor quality or inappropriate, then the immune system could be impaired, increasing R_0 (Becker et al., 2015).
- Foraging behaviour. Reduced foraging behaviour due to the ready availability of food at feeding stations could result in increased aggregation and increased R_0 (Becker et al., 2015).

- Predisposing factors such as S:I:R status, age, sex, and behaviour arising from different demographic sub-populations. Thus supplementary feeding could increase the number of young birds joining and surviving in the population, but in the presence of an infectious agent these immunologically naïve young birds would have the effect of increasing R_0 (Becker et al., 2015). A similar effect could be seen if supplementary feeding allowed infected birds to survive for longer, increasing R_0 . Conversely, supplementary feeding might result in more birds surviving and becoming immune, improving overall flock immunity and reducing R_0 . Accordingly, Becker et al (2015) commented that an understanding of all the demographic processes was essential to predict the end result of host:pathogen interactions.
- Precipitating factors that may finally “tip the scales” such as exposure to another pathogen, the onset of adverse weather conditions, or stress associated with aggression and increased competition for food.

7.2.4 Environmental and host population context

The pathogen factors, species factors and individual bird factors discussed above act within the overall context of the environment and host population. The transmission of many infectious diseases is density-dependent (Roche et al., 2012), and increases in either the degree of environmental contamination or the population density may result in increased contact with the pathogen and greater R_0 . However, not only is the population size or density important, but the nature and diversity of species present may also be important, because different species may be more or less innately susceptible and have different proportions of S:I:R, which results in different contact rates between and within species (Roche et al., 2012). These authors showed that the introduction of a novel susceptible species to the host assemblage could increase pathogen transmission if the introduced species increased overall population density or replaced a less-susceptible species. This “force of infection” will be influenced by the pathogen and host parameters summarised in Tables 7.1 and 7.2.

7.2.5 Positive effects of provisioning on host-pathogen interactions

Although supplementary feeding can have adverse effects as described above, there are also examples where the provision of supplementary feed can have either positive or no effects on disease transmission. Becker et al. (2015) conducted a review and meta-analysis of studies involving food provisioning and disease in wild animals and birds, and found no relationship between supplementary feeding (provisioning) and infection in 65% of studies, an adverse effect in 24%, and a beneficial effect in 11%. They listed some situations where provisioning had a beneficial effect; for example red foxes (*Vulpes vulpes*) feeding on urban waste had less exposure to the tapeworm *Echinococcus multilocularis* because of decreased consumption of the intermediate hosts of the parasite, and long-tailed macaques (*Macaca fascicularis*) had reduced burdens of protozoa such as *Giardia* sp. and *Endolimax* sp. due to reduced exposure and better nutrition. However, these authors cited other examples where provisioning had a deleterious effect, including trichomonosis in greenfinches, brucellosis in elk (*Cervus elephas*) and tuberculosis in white-tailed deer (*Odocoileus virginianus*). These authors also found that hosts foraging on accidental food sources in the urban environment had reduced infection compared with those feeding at urban sites where food had been intentionally provided.

7.3 Contributions of salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in greenfinches, chaffinches, siskins and house sparrows, by time period and region

7.3.1 Overview

A marked decrease in salmonellosis in garden birds was described in Chapter 4, with fewer carcasses, a reduction in the percentage of diagnosable submissions and a fall in salmonellosis incidents. This reduction affected all major species except siskins. A similar fall in carcase numbers, incidents and percentage of diagnosable submissions

was observed for *E. albertii* bacteraemia in siskins and especially greenfinches (Chapter 5); and fluctuations in trichomonosis, including a significant reduction in cases in greenfinches but increase in siskins and redpolls, was discussed in Chapter 6.

However, trying to interpret the trends seen with individual diseases in each species in isolation is likely to be misleading, because in reality mixed infections often occur and the host population will be made up of several different species of bird. In addition, factors such as the population size or density of a species of wild bird in particular time frames or regions could result in bias when interpreting possible disease trends. By the same token, the likelihood that carcasses of certain species or from some regions or time frames might be found or submitted could vary, thus increasing bias. On the other hand, the actual cause of death, be it salmonellosis, *E. albertii* bacteraemia or trichomonosis, should not affect the likelihood that a carcass would be found or submitted. Comparing the *numbers* and *proportions* of the principal causes of death in one species of bird within one region of Scotland over a relatively short time frame should, therefore, help to address this potential bias. In addition, analysis by *species mortality incidents* rather than species carcass numbers removes potential skewing of the data that might arise if large numbers of carcasses were submitted from some incidents. A new “greenfinch mortality incident” was recorded when one or more greenfinch carcasses were submitted and there had been at least three months since other greenfinch carcasses had been submitted from that site. Equivalent criteria were used to define mortality incidents in other species of bird.

Tables 7.3-7.6 and Figures 7.1-7.8 summarise the contributions made by salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in different species of birds.

7.3.2 Contributions of salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in greenfinches

As shown in Table 7.3 and Figure 7.1, salmonellosis was the commonest finding in greenfinch mortality incidents in the north of Scotland in 1994-2003. In 2004-2008, the rise in trichomonosis meant that salmonellosis was no longer as dominant, and in 2009-2013 most greenfinch mortality incidents were caused by trichomonosis. In the south of Scotland trichomonosis had already overtaken salmonellosis by 2004-2008, and in 2009-2013 the great majority of greenfinch mortality incidents in the south of Scotland were caused by trichomonosis (Figure 7.2).

Table 7.3: Causes of greenfinch mortality incidents by time period and region

Time period	Cause of death	Number of incidents (percentage [95% CI])	Number of incidents (percentage [95% CI])
		North	South
1994-2003	Salmonellosis	32 (78.0% [62.0-88.9])	6 (25.0% [10.6-47.0])
	<i>E. albertii</i> bacteraemia	8 (19.5% [9.4-35.4])	8 (33.3% [16.4-55.3])
	Trichomonosis	0 (0.0% [0.0-10.7])	0 (0.0% [0.0-17.2])
	Other	1 (2.4% [1.3-14.4])	11 (45.8% [26.2-66.8])
	Total number of greenfinch mortality incidents*	41	24
2004-2008	Salmonellosis	20 (55.6% [38.3-71.2])	35 (27.3% [20.0-36.0])
	<i>E. albertii</i> bacteraemia	2 (5.6% [1.0-20.0])	5 (3.9% [1.4-9.3])
	Trichomonosis	13 (36.1% [21.3-53.8])	74 (57.8% [48.8-66.4])
	Other	5 (13.9% [5.2-30.3])	19 (14.8% [9.4-22.5])
	Total number of greenfinch mortality incidents*	36	128
2009-2013	Salmonellosis	1 (8.3% [0.4-40.2])	1 (2.4% [0.1-14.4])
	<i>E. albertii</i> bacteraemia	1 (8.3% [0.4-40.2])	1 (2.4% [0.1-14.4])
	Trichomonosis	10 (83.3% [50.9-97.1])	35 (85.4% [70.1-93.9])
	Other	2 (16.7% [2.9-49.1])	5 (12.2% [4.6-27.0])
	Total number of greenfinch mortality incidents*	12	41

*sum of different causes of mortality exceeds the total number of greenfinch mortality incidents because some incidents had more than one cause diagnosed.

Figure 7.1: Greenfinch mortality incidents, by cause and time period – north of Scotland

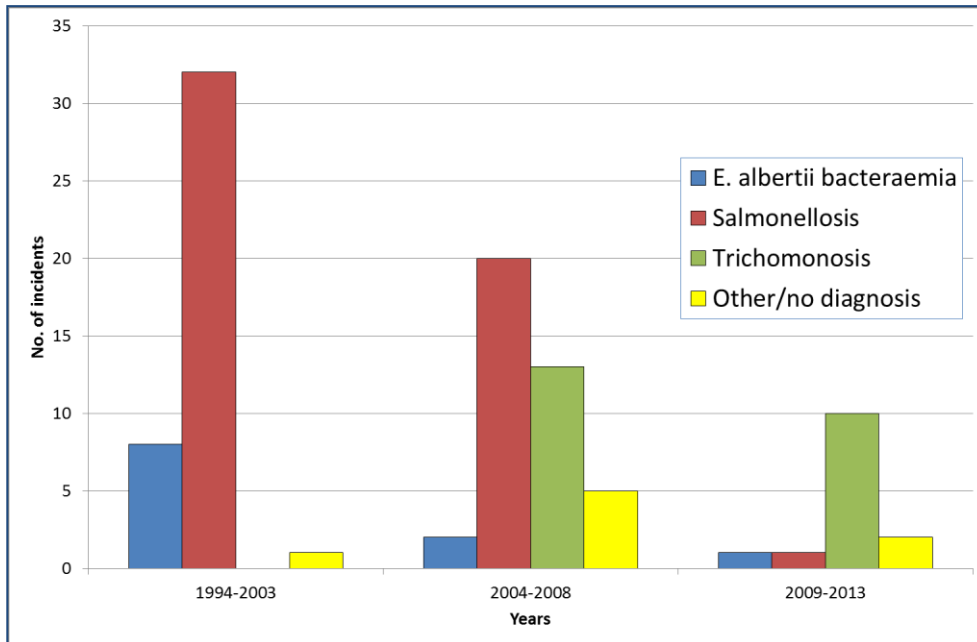
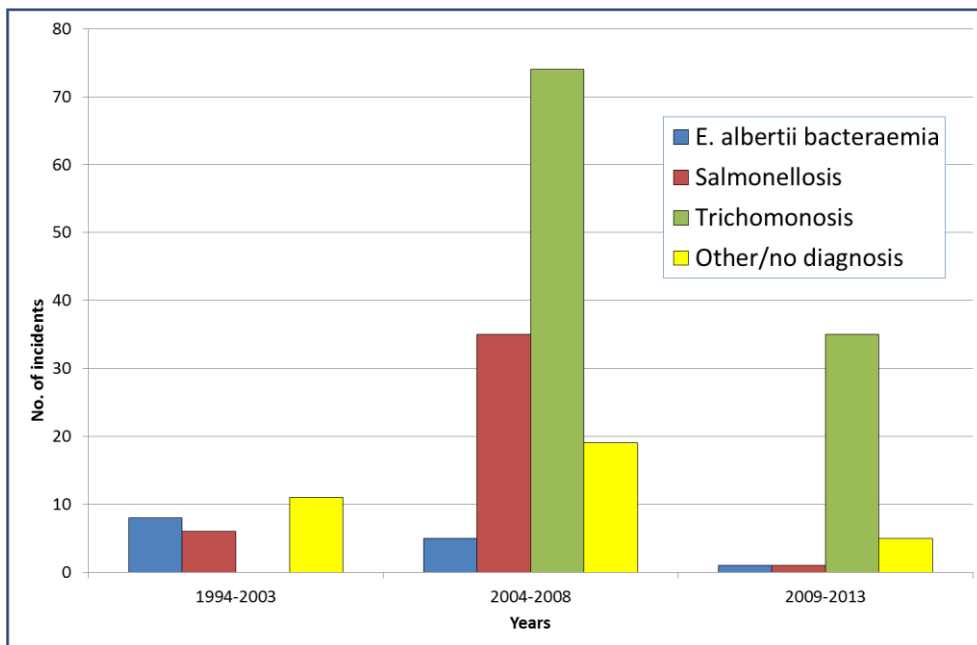


Figure 7.2: Greenfinch mortality incidents, by cause and time period – south of Scotland



7.3.3 Contributions of salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in chaffinches

The changing significance of salmonellosis and trichomonosis in chaffinch mortality incidents in the north and south of Scotland was very similar to that seen in greenfinches, although other diagnoses were also common (Table 7.4, Figures 7.3 and 7.4). In the north, salmonellosis was the commonest infectious cause of chaffinch mortality incidents in 1994-2003, by 2004-2008 there were similar numbers of incidents due to salmonellosis and trichomonosis, and in 2009-2013 trichomonosis far outnumbered other causes of chaffinch mortality incidents. In the south, although salmonellosis predominated in 1994-2003, thereafter trichomonosis was the dominant infectious cause of chaffinch mortality incidents.

Table 7.4: Causes of chaffinch mortality incidents by time period and region

Time period	Cause of death	Number of incidents (percentage [95% CI])	Number of incidents (percentage [95% CI])
		North	South
1994-2003	Salmonellosis	3 (60.0% [17.0-92.7])	6 (35.3% [15.3-61.4])
	<i>E. albertii</i> bacteraemia	0 (0.0% [0.0-53.7])	1 (5.9% [0.3-30.8])
	Trichomonosis	0 (0.0% [0.0-53.7])	0 (0.0% [0.0-22.9])
	Other	3 (60.0% [17.0-92.7])	13 (76.5% [49.8-92.2])
	Total number of chaffinch mortality incidents*	5	17
2004-2008	Salmonellosis	11 (39.3% [22.1-59.3])	15 (18.3% [10.9-28.7])
	<i>E. albertii</i> bacteraemia	2 (7.1% [1.2-24.9])	2 (2.4% [0.4-9.3])
	Trichomonosis	10 (35.7% [19.3-55.9])	42 (51.2% [40.0-62.3])
	Other	10 (35.7% [19.3-55.9])	30 (36.6% [26.4-48.0])
	Total number of chaffinch mortality incidents*	28	82
2009-2013	Salmonellosis	0 (0.0% [0.0-21.9])	1 (2.5% [0.1-14.7])
	<i>E. albertii</i> bacteraemia	0 (0.0% [0.0-21.9])	1 (2.5% [0.1-14.7])
	Trichomonosis	15 (83.3% [57.7-95.6])	29 (72.5% [55.9-84.9])
	Other	7 (38.9% [18.3-63.9])	13 (32.5% [19.1-49.2])
	Total number of chaffinch mortality incidents*	18	40

*sum of different causes of mortality exceeds the total number of chaffinch mortality incidents because some incidents had more than one cause diagnosed.

Figure 7.3: Chaffinch mortality incidents, by cause and time period – north of Scotland

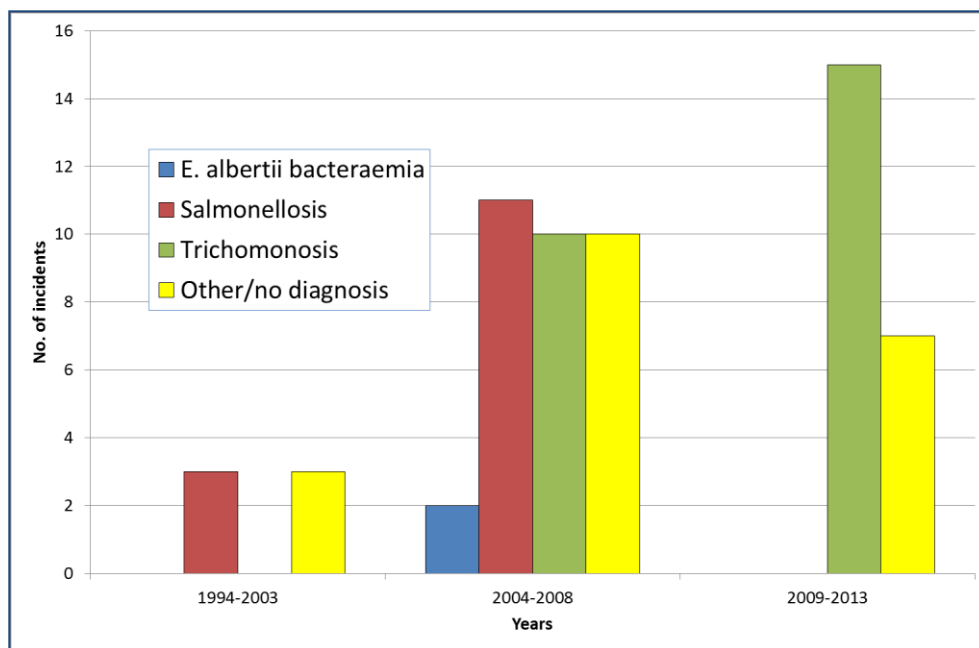
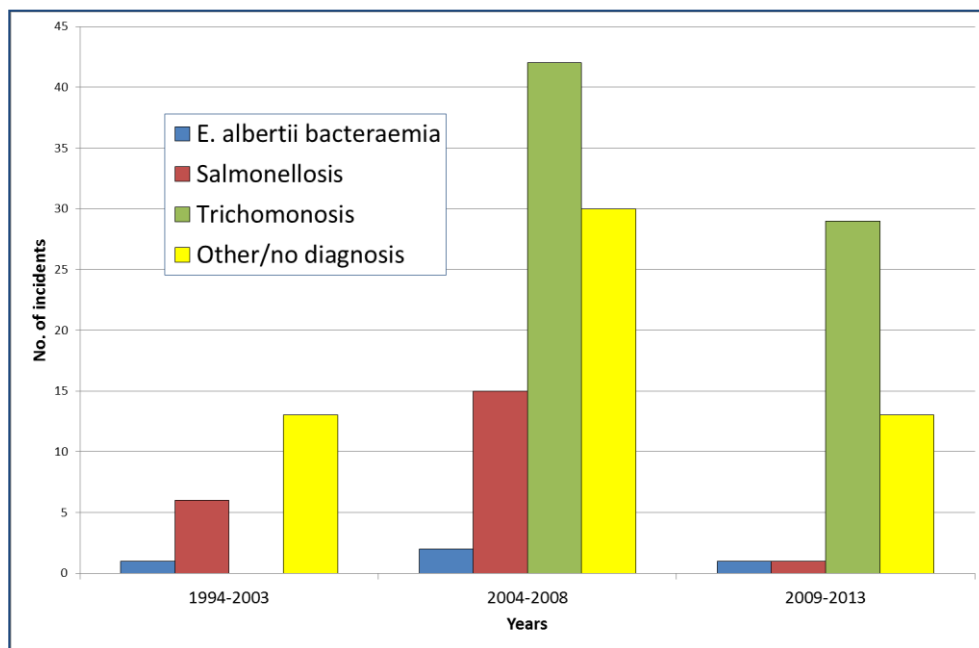


Figure 7.4: Chaffinch mortality incidents, by cause and time period – south of Scotland



7.3.4 Contributions of salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in siskins

In contrast to the situation observed in greenfinches and chaffinches, the commonest cause of siskin mortality incidents in 1994-2003 in both the north and south of Scotland was *E. albertii* bacteraemia. This continued into 2004-2008 in both regions, although with increasing numbers of incidents caused by salmonellosis. By 2009-2013, salmonellosis, *E. albertii* bacteraemia and trichomonosis contributed to siskin mortality incidents to broadly similar extents (Table 7.5, Figures 7.5 and 7.6).

Table 7.5: Causes of siskin mortality incidents by time period and region

Time period	Cause of death	Number of incidents (percentage [95% CI])	Number of incidents (percentage [95% CI])
		North	South
1994-2003	Salmonellosis	1 (11.1% [0.6-49.3])	1 (4.5% [0.2-24.9])
	<i>E. albertii</i> bacteraemia	8 (88.9% [50.7-99.4])	15 (68.2% [45.1-85.3])
	Trichomonosis	0 (0.0% [0.0-37.1])	0 (0.0% [0.0-18.5])
	Other	0 (0.0% [0.0-37.1])	6 (27.3% [11.6-50.4])
	Total number of siskin mortality incidents	9	22
2004-2008	Salmonellosis	6 (24.0% [10.2-45.5])	8 (22.9% [11.0-40.6])
	<i>E. albertii</i> bacteraemia	15 (60.0% [38.9-78.2])	15 (42.9% [26.8-60.5])
	Trichomonosis	0 (0.0% [0.0-16.6])	3 (8.6% [2.2-24.2])
	Other	4 (16.0% [5.2-36.9])	9 (25.7% [13.1-43.6])
	Total number of siskin mortality incidents	25	35
2009-2013	Salmonellosis	6 (37.5% [16.3-64.1])	9 (36.0% [18.7-57.4])
	<i>E. albertii</i> bacteraemia	5 (31.2% [12.1-58.5])	6 (24.0% [10.2-45.5])
	Trichomonosis	4 (25.0% [8.3-52.6])	8 (32.0% [15.7-53.5])
	Other	2 (12.5% [2.2-39.6])	4 (16.0% [5.2-36.9])
	Total number of siskin mortality incidents*	16	25

*sum of different causes of mortality exceeds the total number of siskin mortality incidents because some incidents had more than one cause diagnosed.

Figure 7.5: Siskin mortality incidents, by cause and time period – north of Scotland

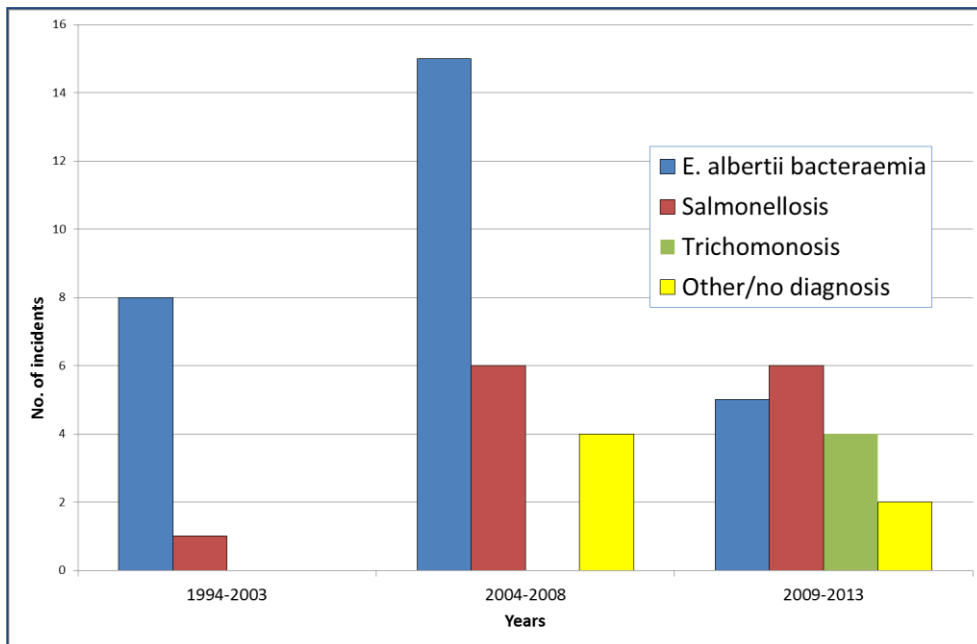
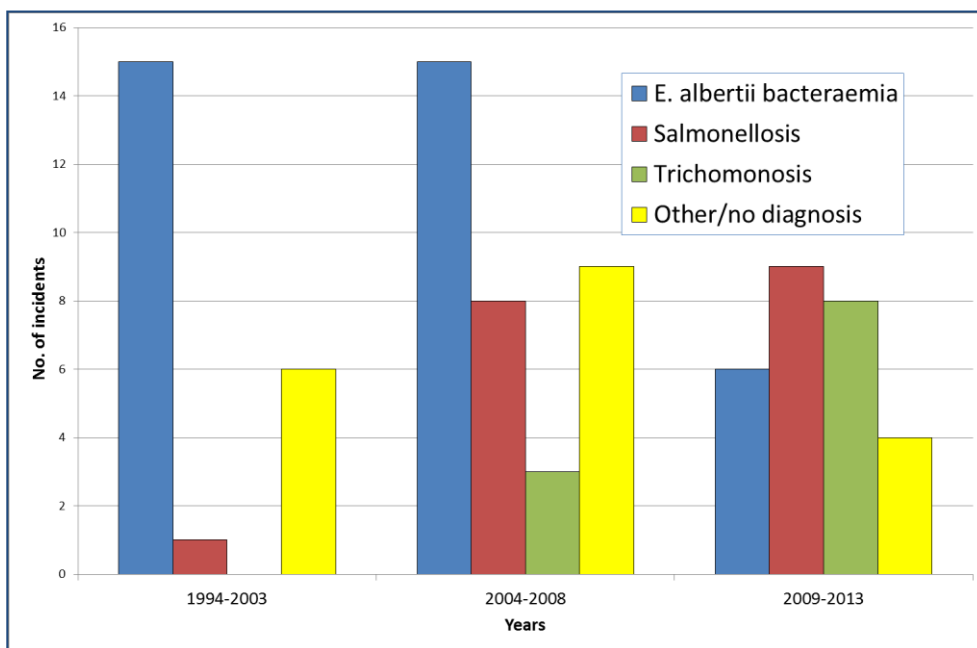


Figure 7.6: Siskin mortality incidents, by cause and time period – south of Scotland



7.3.5 Contributions of salmonellosis, *E. albertii* bacteraemia and trichomonosis to mortality incidents in house sparrows

No cases of *E. albertii* bacteraemia, and very few cases of trichomonosis, were seen in house sparrows. The trend was for a reduction in mortality incidents caused by salmonellosis, but relatively low numbers of house sparrow mortality incidents, especially in the north of Scotland, precluded meaningful interpretation (Table 7.6, Figures 7.7 and 7.8).

Table 7.6: Causes of house sparrow mortality incidents by time period and region

Time period	Cause of death	Number of incidents (percentage [95% CI])	Number of incidents (percentage [95% CI])
		North	South
1994-2003	Salmonellosis	2 (66.7% [12.5-98.2])	12 (60.0% [36.4-80.0])
	<i>E. albertii</i> bacteraemia	0 (0.0% [0.0-69.0])	0 (0.0% [0.0-20.0])
	Trichomonosis	0 (0.0% [0.0-69.0])	0 (0.0% [0.0-20.0])
	Other	1 (33.3% [1.8-87.5])	8 (40.0% [20.0-63.6])
	Total number of house sparrow mortality incidents	3	20
2004-2008	Salmonellosis	3 (42.9% [11.8-79.8])	10 (40.0% [21.8-61.1])
	<i>E. albertii</i> bacteraemia	0 (0.0% [0.0-43.9])	0 (0.0% [0.0-16.6])
	Trichomonosis	0 (0.0% [0.0-43.9])	1 (4.0% [0.2-22.3])
	Other	4 (57.1% [20.2-88.2])	15 (60.0% [38.9-78.2])
	Total number of house sparrow mortality incidents*	7	25
2009-2013	Salmonellosis	1 (33.3% [1.8-87.5])	5 (31.2% [12.1-58.5])
	<i>E. albertii</i> bacteraemia	0 (0.0% [0.0-69.0])	0 (0.0% [0.0-24.1])
	Trichomonosis	1 (33.3% [1.8-87.5])	1 (6.2% [0.3-32.3])
	Other	3 (100.0% [31.0-100.0])	11 (68.7% [41.5-87.9])
	Total number of house sparrow mortality incidents *	3	16

*sum of different causes of mortality exceeds the total number of house sparrow mortality incidents because some incidents had more than one cause diagnosed.

Figure 7.7: House sparrow mortality incidents, by cause and time period – north of Scotland

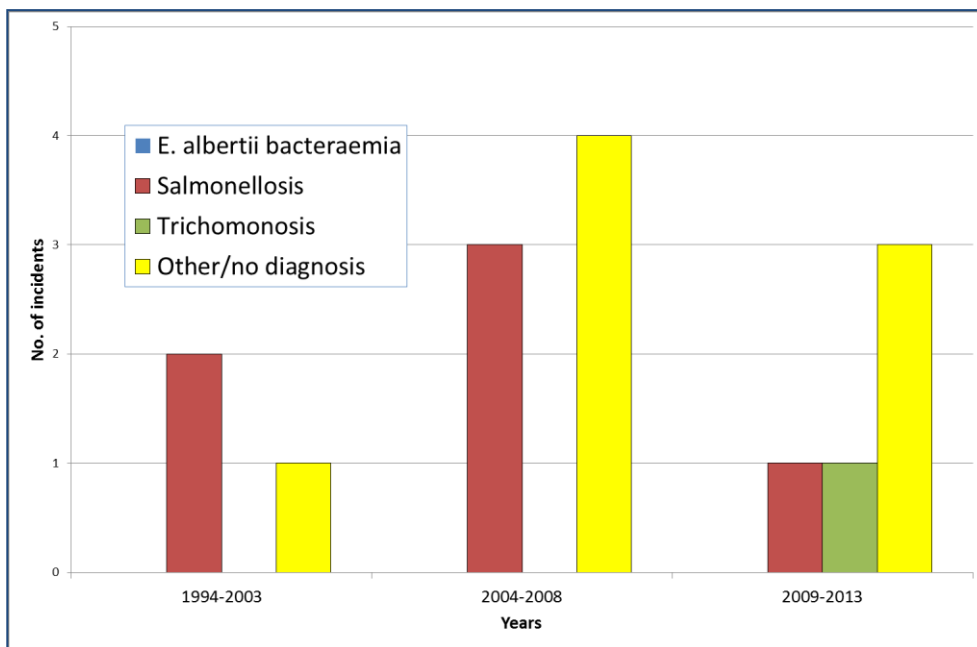
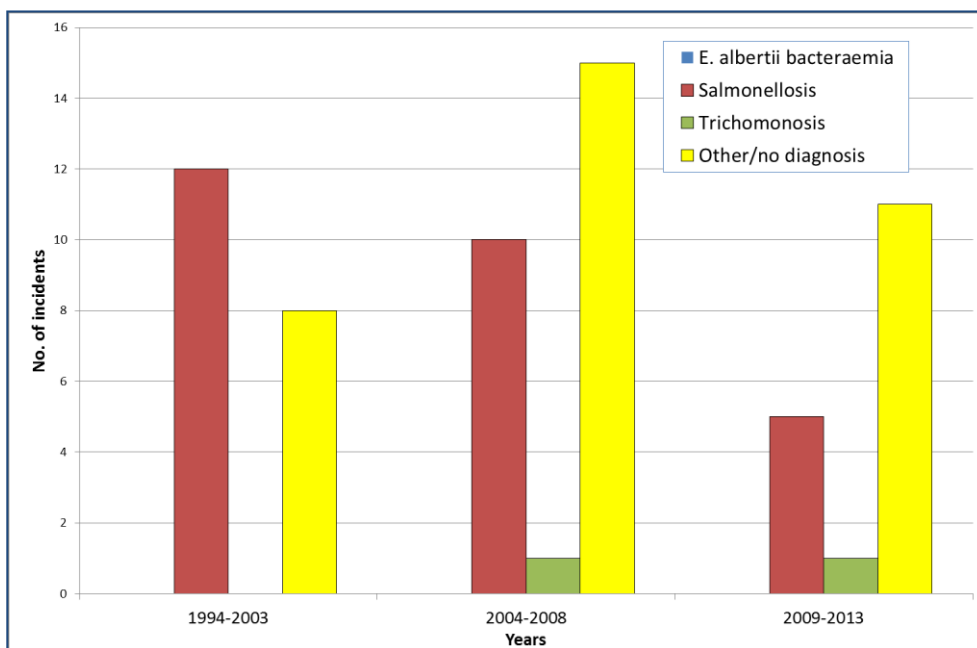


Figure 7.8: House sparrow mortality incidents, by cause and time period – south of Scotland



7.3.6 The changing balance of salmonellosis, *E. albertii* bacteraemia and trichomonosis

Figures 7.1-7.8 and Tables 7.3-7.6 summarised the fluctuations in the causes of species mortality incidents from 1994 to 2013. In the first half of the study period (1994-2003), salmonellosis was the commonest cause of death recorded in mortality incidents involving greenfinches, chaffinches and house sparrows in the north of Scotland, and in chaffinch and house sparrow mortality incidents in the south. *E. albertii* bacteraemia and salmonellosis were equally represented in greenfinches in the south, and *E. albertii* bacteraemia predominated in siskin incidents in both regions during the first ten years of the study.

The emergence of trichomonosis in garden birds from 2005 substantially changed the patterns of disease observed in small garden birds, especially greenfinches. In the south of Scotland, trichomonosis became the dominant disease diagnosed in greenfinches and chaffinches between 2004 and 2008, and by 2009-2013 almost all cases of infectious disease in these species were the result of trichomonosis, with very few diagnoses of salmonellosis or *E. albertii* bacteraemia. Trichomonosis took a little longer to become established in the north, but by 2009-2013 the situation in greenfinch and chaffinch mortality incidents in the north reflected that seen in the south of Scotland. House sparrows were not affected by *E. albertii* bacteraemia and only infrequently by trichomonosis, and salmonellosis remained the most important infectious disease in this species, especially in the south of Scotland. Nevertheless, the number of house sparrow mortality incidents caused by salmonellosis fell during the study, although the number of recorded mortality incidents was low. The opposite trend was seen in siskin mortality incidents, with salmonellosis responsible for an increasing proportion of incidents in the second half of the surveillance period.

One reason for the overall reduction of salmonellosis and *E. albertii* bacteraemia in finch mortality incidents may have been the natural epidemic/endemic cycle, with a reduction in cases as the proportion of susceptible birds (S) decreased and recovered

birds (R) increased, resulting in fewer infectious birds (I) and a reduction in R_0 . A further factor was likely to be the rise in trichomonosis in greenfinches and a corresponding reduction in the greenfinch population (Lawson et al., 2012b), reducing the number of susceptible birds and density-dependent transmission between greenfinches. In addition, if greenfinches are a reservoir host for some garden bird phage types of *S. Typhimurium*, as proposed by Pennycott et al. (1998) and Lawson et al. (2010), a reduction in greenfinch numbers would also have resulted in fewer inter-species contacts by infectious birds and thus fewer cases of salmonellosis in other species such as chaffinches and goldfinches.

The trend for a reduction of salmonellosis in house sparrows, which are less commonly affected by trichomonosis, could similarly have been part of the natural epidemic/endemic cycle or the indirect result of the diminished reservoir of salmonellosis in greenfinches. However, the decline in salmonellosis in house sparrows occurred from 2001, prior to the finch trichomonosis epidemic, and is more likely to have been a direct reflection of the overall reduction in house sparrow numbers observed in recent years (Hole et al., 2002). It was proposed that the decline in rural house sparrows was caused by changes in farming practice reducing winter food supplies, and a reduction in invertebrates and insufficient nest sites adversely affected breeding performance of house sparrows in towns and cities (Gillings and Balmer, 2013). Whatever the cause, the decline in the house sparrow population will have reduced the number of susceptible house sparrows likely to succumb to salmonellosis. A correlation between house sparrow numbers using a garden bird feeding station and contamination of the feeding station with *S. Typhimurium* was reported by Pennycott et al. (2005a); therefore the national reduction in house sparrow numbers could also have reduced the likelihood of salmonellosis in other birds at feeding stations.

Trichomonosis in chaffinches and greenfinches showed a typical epidemic pattern, with case numbers increasing rapidly, most likely due to the high proportion of susceptible birds (S) in the population and high R_0 , then reducing and becoming endemic as the

number of recovered birds (R) exceeded the number of S. The rapid spread of disease could not only reflect the large population of susceptible finches present in 2005 (Musgrove et al., 2010), but also the ease of transfer of the organism within and between adults and young birds. Greenfinches may have been at greater risk than chaffinches for the reasons described in 7.2 and Table 7.2, facilitating the transfer of trichomonads among greenfinches. Trichomonosis did not disappear from the population, partly because current-year birds provided new susceptible birds each year, and partly because the number of infectious birds remained high due to non-clinical carriage and transmission by some adults when feeding their young or during courtship.

Unlike the trends seen in most other species examined, there was a rise in salmonellosis in siskin mortality incidents in the second half of the surveillance period and an increase in trichomonosis in the final five years. This most likely reflected the increased number of siskins using garden feeding stations in recent years (BTO 2013a), increasing the size of the susceptible population. The increase in salmonellosis and trichomonosis in siskins in the final five years occurred at a time when incidents of *E. albertii* bacteraemia fell in that species, suggesting that the proportions of S:I:R in the siskin population varied for each different disease.

A similar trend was also noted in lesser redpolls, in which cases of salmonellosis were confined to the final four years of the study (Figure 4.11) and trichomonosis was only seen in the final year (Figure 6.11). Lesser redpolls frequently feed alongside siskins and since 2008 there has been a 15-fold increase in the number of reports of lesser redpolls using garden feeding stations (BTO 2013b).

Overall, this 20-year study has shown that patterns of infectious diseases such as salmonellosis, *E. albertii* bacteraemia and trichomonosis have changed over time, with interaction between the effects of the different diseases. These patterns are likely to change again as new factors come into play. As noted by Roche et al. (2012), the introduction of a novel and susceptible host species (for example siskins and redpolls)

can increase pathogen transmission by increasing population density and by replacing less-susceptible individuals and species with more-susceptible birds. This aspect of attracting multiple species of birds to feeding stations is further discussed in 7.7.

7.4 Monthly distribution of carcasses

The seasonal submissions of carcasses with salmonellosis, *E. albertii* bacteraemia and trichomonosis are summarised in Table 7.7 (north of Scotland) and 7.8 (south of Scotland). The figures in blue in these tables indicate months in which 10-15% of the total diagnoses of that condition occurred, and months in which over 15% of diagnoses occurred are indicated in red. Substantial variation between seasonal patterns for different diseases in the same species of bird in the same region is apparent.

The peak months in which different species of bird are likely to be present in gardens (expressed as the percentage of gardens reporting the presence of that species to the BTO Garden BirdWatch (<http://blx1.bto.org/gbw-dailyresults/results/> accessed 02/04/15) are shown in Table 7.9.

Figures 7.9-7.12 show the monthly distribution of different conditions in different species in the south of Scotland, with the peak BTO reporting months highlighted. Comparisons have been restricted to the south of Scotland, where greater carcass numbers and diversity of diseases were recorded.

Table 7.7: Different conditions by month – north of Scotland

Condition	Number of carcasses	Months of year in which 10% or more of total diagnoses occurred (10% - 15% shown in blue, over 15% in red)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Salmonellosis in greenfinches	97	35	39	14									
Salmonellosis in chaffinches	21	43	52										
Salmonellosis in siskins	23	22	26	18			26						
<i>E. albertii</i> in siskins	55				18	38	15	25					
<i>E. albertii</i> in greenfinches	15				46	27							
Trichomonosis in greenfinches	39						21		13			15	20
Trichomonosis in chaffinches	46	18				17			13			13	

Table 7.8: Different conditions by month – south of Scotland

Condition	Number of carcasses	Months of year in which 10% or more of total diagnoses occurred (10% - 15% shown in blue, over 15% in red)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Salmonellosis in greenfinches	57	44	19									12	18
Salmonellosis in chaffinches	29	55	24	14									
Salmonellosis in siskins	23	13	22	52									
Salmonellosis in house sparrows	46	34	14									11	14
<i>E. albertii</i> in siskins	53				41	32							
<i>E. albertii</i> in greenfinches	17			18	41	23							
Trichomonosis in greenfinches	139							22	22	20		12	
Trichomonosis in chaffinches	99					11			20	19			
Trichomonosis in siskins	13					38	23						

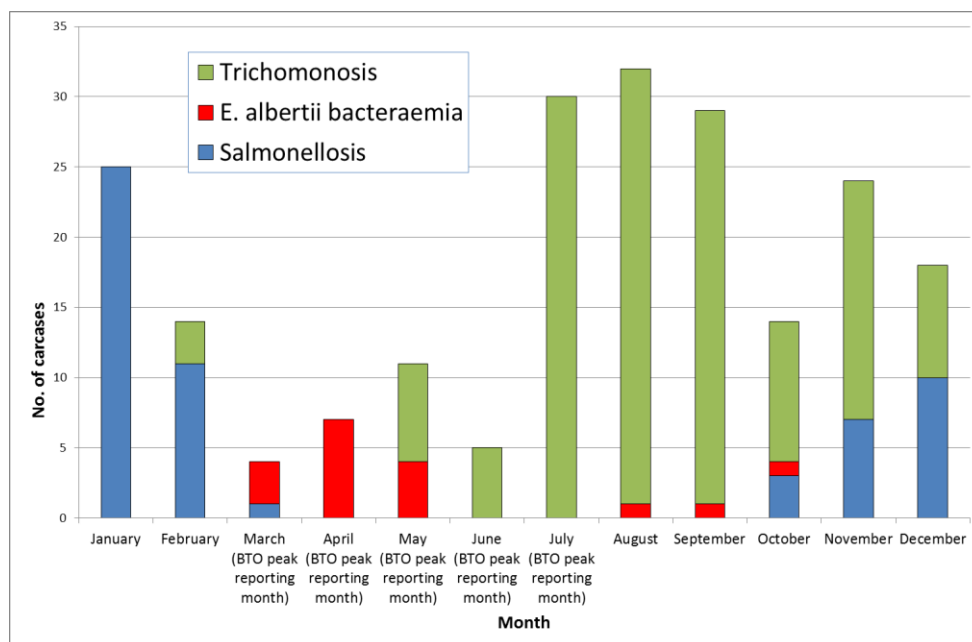
Table 7.9: Percentages of gardens reporting different species of birds, by region and month.

Species	Region	Percentage of gardens reporting birds (peak months are shown in red).											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Greenfinch	North	70	70	75	75	80	75	75	70	65	65	65	70
Greenfinch	South	65	70	75	75	80	75	75	70	65	60	60	65
Chaffinch	North	90	90	90	90	90	80	80	75	75	80	80	85
Chaffinch	South	90	90	90	90	90	80	80	80	75	80	80	85
Goldfinch	North	30	30	30	35	40	30	30	20	15	20	20	25
Goldfinch	South	40	40	40	50	55	40	40	25	25	25	30	35
Siskin	North	30	35	40	50	55	45	45	35	20	15	10	10
Siskin	South	25	30	35	40	40	25	30	20	10	10	10	10
House sparrow	North	70	70	70	75	75	80	80	80	75	75	75	75
House sparrow	South	65	65	70	70	75	80	80	75	70	70	70	70
Dunnock	North	80	80	80	75	70	65	65	60	55	70	75	80
Dunnock	South	85	85	85	85	80	75	75	75	70	75	80	80

Data from BTO Garden BirdWatch, <http://blx1.bto.org/gbw-dailyresults/results/> Accessed 02/04/15.

Broadly speaking, salmonellosis in greenfinches in the south of Scotland was most commonly diagnosed in January to February and November to December, but *E. albertii* bacteraemia in greenfinches was mostly seen in March to May, and trichomonosis in greenfinches was especially common in July to September (Table 7.8, Figure 7.9). However, the peak reporting rates for greenfinches in gardens in the south of Scotland occurred in March to July (Table 7.9, Figure 7.9).

Figure 7.9: Different conditions and BTO peak reporting rates – greenfinches in south of Scotland



Similar differences between disease occurrence and peak reporting rates were found for chaffinches, siskins and house sparrows. Salmonellosis in chaffinches in the south of Scotland was most frequently diagnosed in January to March, but trichomonosis was more spread out and with peaks in May, August and September (Table 7.8, Figure 7.10). In contrast, peak reporting rates for chaffinches were January to May (Table 7.9, Figure 7.10). Salmonellosis in siskins in the south was mostly in January to March, *E. albertii* bacteraemia peaked in April and May, while trichomonosis in siskins was commonest in May and June (Table 7.8, Figure 7.11), but peak reporting rates for siskins in gardens in the south of Scotland were in March to May (Table 7.9, Figure 7.11). Salmonellosis in house sparrows peaked in November to February (Table 7.8, Figure 7.12), but peak reports of house sparrows in gardens were in June to August (Table 7.9, Figure 7.12).

Figure 7.10: Different conditions and BTO peak reporting rates – chaffinches in south of Scotland

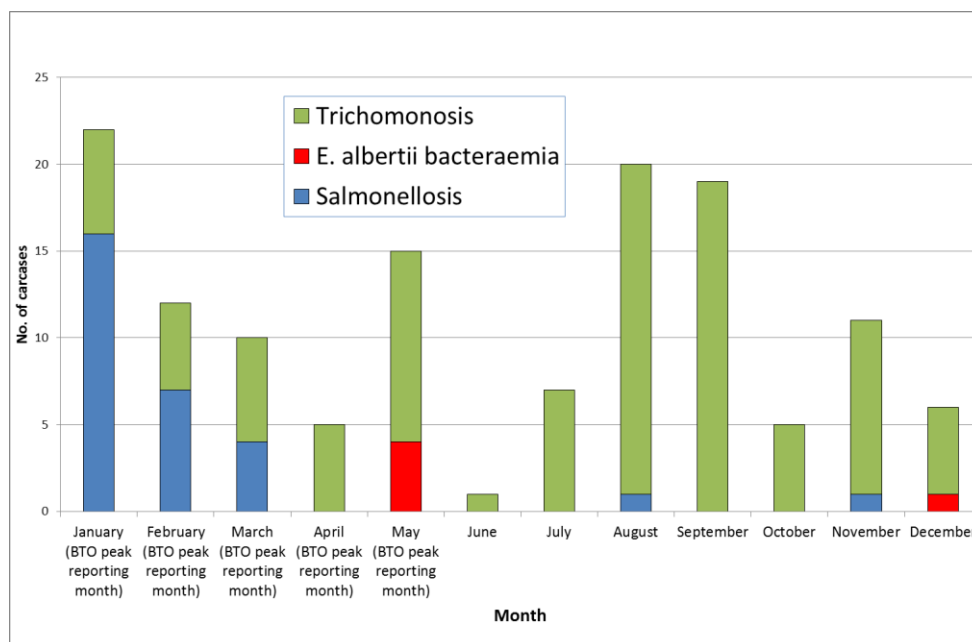


Figure 7.11: Different conditions and BTO peak reporting rates – siskins in south of Scotland

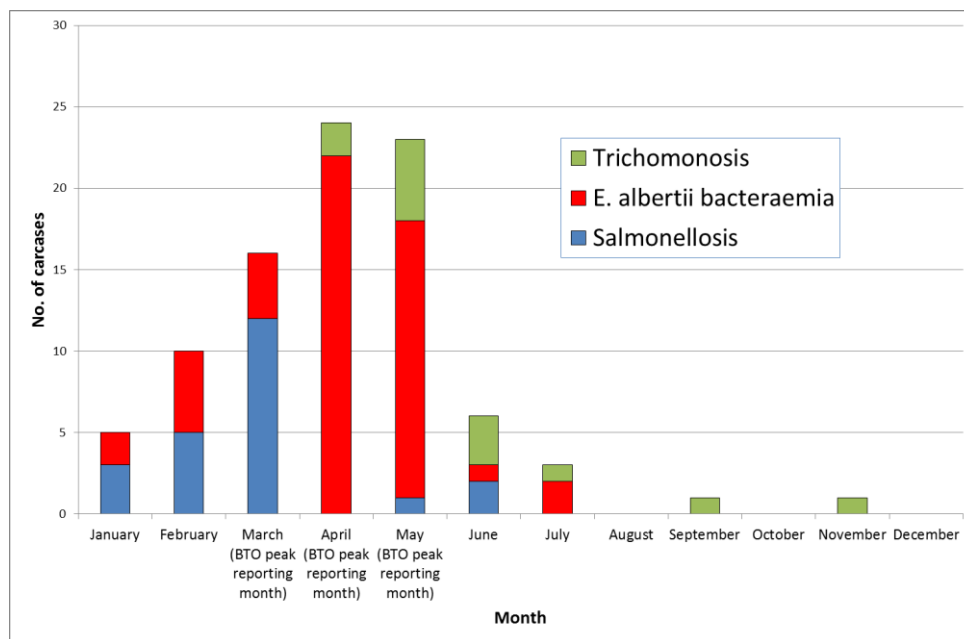
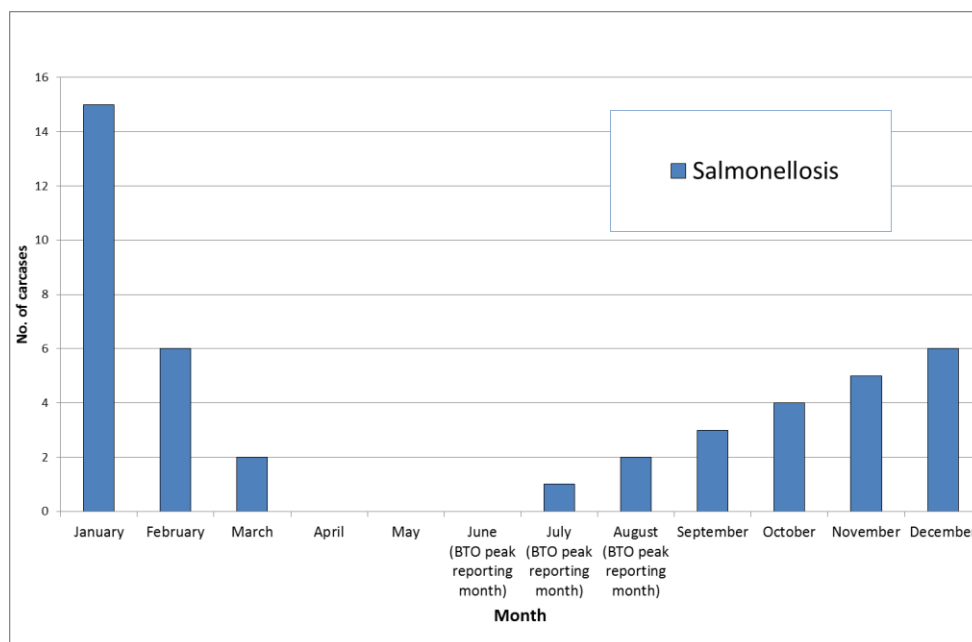


Figure 7.12: Salmonellosis and BTO peak reporting rates – house sparrows in south of Scotland



Density-dependent disease transmission is undoubtedly important in the spread of salmonellosis, *E. albertii* bacteraemia and trichomonosis. Nevertheless, it is clear that the seasonal disease patterns did not solely reflect the likelihood of birds of a particular species being present in gardens in different months, otherwise the monthly patterns for the different diseases would have been similar. By the same token, if the total number of birds of one or more species (rather than presence/absence) was the sole driving force of infection, the impact would have been the same for all three diseases, rather than producing the different patterns observed. Other factors as outlined in 7.2 above are, therefore, likely to have been superimposed on density-dependent transmission driven by population density, and two important factors, sex and age of affected birds, are discussed further in 7.5 and 7.6 below.

7.5 Sex ratio

Approximately three-quarters of the deaths in adult birds from salmonellosis and *E. albertii* bacteraemia were recorded in male birds, as were two-thirds of deaths from trichomonosis (Chapters 4-6). Males outnumbered females to a statistically significant extent for salmonellosis in greenfinches and chaffinches, *E. albertii* bacteraemia in siskins, and trichomonosis in greenfinches, chaffinches and goldfinches (Table 7.10). The same trend was seen for salmonellosis in goldfinches, siskins and house sparrows, and *E. albertii* bacteraemia in greenfinches, but the differences were not statistically significant although possibly biologically significant.

Table 7.10: Sex ratio of adult birds with salmonellosis, *E. albertii* bacteraemia and trichomonosis

Condition	Overall ratio (M:F), p value*	Month of year in which adult sex ratio equaled or exceeded 3:1 (dominant sex highlighted)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Salmonellosis in greenfinches	3.9:1 (97:25) p<0.001	M	M									M	
Salmonellosis in chaffinches	2.3:1 (30:13) p=0.029	M		M									
Salmonellosis in goldfinches	2.2:1 (11:5) p=0.163												
Salmonellosis in siskins	1.9:1 (25:13) p=0.096	M	M										
Salmonellosis in house sparrows	1.7:1 (24:14) p=0.166												
<i>E. albertii</i> in siskins	3.7:1 (67:18) p<0.001		M	M	M	M		M					
<i>E. albertii</i> in greenfinches	2.0:1 (16:8) p=0.142												
Trichomonosis in greenfinches	2.0:1 (88:43) p=0.008					F					M	M	M
Trichomonosis in chaffinches	2.5:1 (83:33) p=0.001		M	M			F	M	M	M	M	M	
Trichomonosis in goldfinches	(9:0) p=0.004											M	
Trichomonosis in siskins	0.9:1 (6:7) p=0.794						F						

*p value for two-tailed binomial test, assuming a theoretical population made up of 50% males and 50% females.

Table 7.10 also presents the months in which males outnumbered females by a factor of three or more. For salmonellosis, male-dominant months were most often January and February, for *E. albertii* bacteraemia in siskins the main block of male dominance was February to May, and for trichomonosis male dominance tended to be later in the year, especially July to December. There were also some months in which females outnumbered males by a factor of three or more; trichomonosis in greenfinches (May), trichomonosis in chaffinches (June) and trichomonosis in siskins (June). For comparison, deaths from trauma are shown in Table 7.11; taking the twelve months together, male and female chaffinches and house sparrows were affected to an equal extent, but males outnumbered females for trauma in greenfinches, goldfinches and siskins. Only in siskins with trauma was the male dominance statistically significant, but in all three species the differences were probably biologically significant. When examined by month, female greenfinches and chaffinches with trauma outnumbered males in January, but male siskins, goldfinches and greenfinches outnumbered females in February, March and April respectively.

Table 7.11: Sex ratio of adult birds with trauma

Condition	Overall ratio (M:F), p value*	Month of year in which adult sex ratio equaled or exceeded 3:1 (dominant sex highlighted)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Trauma in greenfinches	2.8:1 (14:5) p=0.058	F			M							M	
Trauma in chaffinches	1.1:1 (31:28) p=0.757	F											
Trauma in goldfinches	6.0:1 (6:1) p=0.067			M									
Trauma in house sparrows	1.0:1 (3:3) p=1.000												
Trauma in siskins	9.0:1 (9:1) p=0.016		M										

*p value for two-tailed binomial test, assuming a theoretical population made up of 50% males and 50% females.

A higher preponderance of males could in theory reflect a greater proportion of males visiting feeding stations, behavioural or physiological factors making males more likely

to be exposed to pathogens or suffer from the effects of pathogens, or increased likelihood of carcasses of males being found due to the brighter colours of their plumage (Lawson et al., 2010). However, if caused solely by the ratio of sexes visiting the site or the ease of finding male carcasses, one would expect similar seasonal patterns of sex ratios regardless of the condition being considered. That was not the case in the current analysis, suggesting that the greater involvement of male birds in certain months reflected a combination of the epidemiology of the disease in question and male behavioural and physiological factors.

One physiological factor to be considered is the effect of testosterone levels in male birds. Wilson et al. (2001) highlighted that male vertebrates (including mammals and birds) tended to have higher levels of parasitism and disease than females, and noted that testosterone depressed the humoral and cell-mediated immune systems. Male birds that have high testosterone levels may, therefore, have poorer immune systems and be more susceptible to infectious disease. High levels of testosterone could explain the over-representation of salmonellosis in male finches in January to March, as the birds come into breeding condition. Similarly, the domination of *E. albertii* bacteraemia in male siskins in February to May and male chaffinches with trichomonosis in February and March could be partially explained by high testosterone levels in males in breeding condition. In the case of salmonellosis, if *S. Typhimurium* in wild birds behaves like *S. Pullorum* in poultry, this greater susceptibility of male birds due to elevated testosterone levels is occurring at a time when high levels of circulating sex hormones in females are resulting in re-activation of latent infections of the spleen and subsequent increased horizontal and vertical excretion (Lister and Barrow, 2008).

However, trichomonosis was also a feature in male chaffinches from July to November, was highest in male greenfinches from October to December, and highest in male goldfinches in November. Elevated testosterone levels in male birds post-breeding are unlikely to be the explanation for the predominance of males in these months, and other factors are therefore likely to be involved. Male dominance for salmonellosis and *E.*

albertii bacteraemia was not a major finding in these months, suggesting that the male domination in trichomonosis in the latter half of the year was a specific function of the pathogen or condition itself. It is also interesting to note that for trichomonosis, but not salmonellosis or *E. albertii* bacteraemia, there were some months in which females outnumbered males by a factor of three or more – greenfinches in May and chaffinches and siskins in June – again suggesting that the sex ratio for trichomonosis was not driven by testosterone levels alone.

Behavioural differences could also influence the sex ratio seen in some conditions; deaths from trauma in female greenfinches and chaffinches predominated in January, but traumatic deaths in male siskins, male goldfinches and male greenfinches predominated in February, March and April respectively, suggesting different behavioural patterns in males and females of different species in the pre-breeding season.

In summary, the observed sex biases are probably the end result of a combination of hormonal factors, pathogen factors and behavioural factors, each with varying impact depending on the condition in question. Awareness of the importance of these factors helps to explain the overall seasonal trends for some diseases. Thus if testosterone levels are important in driving salmonellosis in male finches, coupled with greater excretion by females with high levels of sex hormones, more cases of salmonellosis will be seen in the immediate pre-breeding and breeding seasons, as observed in this study. Equally, the unidentified factor or factors that favour trichomonosis in male finches after the breeding season has ended will be partially responsible for the seasonal pattern of this disease. Wilson et al. (2001) commented that different mechanisms causing sex bias often co-vary, making it difficult to disentangle the importance of each factor, and the results of the current analysis support that view.

7.6 Age of birds

Salmonellosis was seen infrequently in immature birds (Chapter 4), but occurred more often in siskins with *E. albertii* bacteraemia in the north of Scotland (Chapter 5) and in some species with trichomonosis (Chapter 6). Depending on the nature of the pathogen, young birds could become infected before hatching (trans-ovarian spread or through faecal contamination of the egg shell), from the nest environment (faecal contamination from nest-mates or adults), when being fed by their parents as nestlings (food material or adult crop contents), or after fledging (when still being fed by parents, and from environmental contamination). Different routes of infection could account for some of the variation noted when considering the age of bird affected by different conditions in different regions (Table 7.12).

Table 7.12: Percentages of birds with different conditions that were immature

	Region	Number of birds in which age/sex identified	Number (and percentage) with the condition that were immature
Greenfinches with salmonellosis	North	78	4 (5.1%)
Greenfinches with salmonellosis	South	47	1 (2.1%)
Chaffinches with salmonellosis	North	20	0 (0.0%)
Chaffinches with salmonellosis	South	23	0 (0.0%)
Siskins with salmonellosis	North	19	1 (5.3%)
Siskins with salmonellosis	South	20	0 (0.0%)
House sparrows with salmonellosis	South	32	1 (3.1%)
Greenfinches with trichomonosis	North	31	3 (9.7%)
Greenfinches with trichomonosis	South	128	26 (20.3%)
Chaffinches with trichomonosis	North	43	5 (11.6%)
Chaffinches with trichomonosis	South	97	19 (19.6%)
Siskins with trichomonosis	North	5	2 (40.0%)
Siskins with trichomonosis	South	11	1 (9.1%)
Siskins with <i>E. albertii</i> bacteraemia	North	51	10 (19.6%)
Siskins with <i>E. albertii</i> bacteraemia	South	44	0 (0.0%)
Greenfinches with <i>E. albertii</i> bacteraemia	North	11	0 (0.0%)
Greenfinches with <i>E. albertii</i> bacteraemia	South	13	0 (0.0%)

For greenfinches, siskins and house sparrows with salmonellosis, in which age and sex were established, only 2-5% were immature birds, and no cases of salmonellosis were diagnosed in immature chaffinches. No significant differences were found between birds of the same species in different regions. Similar findings were made by Lawson et al. (2010), who reported that all cases of salmonellosis diagnosed in garden birds in England and Wales from 1993 to 2003 occurred in adult birds or first-year birds after their juvenile body moult. In this respect, salmonellosis in garden birds behaves like salmonellosis in poultry caused by *S. Gallinarum* (fowl typhoid), in which most deaths occur in mature birds, rather than infection with *S. Pullorum* (pullorum disease), in which most deaths occur in chicks (Lister and Barrow, 2008). Needless to say, the absence of disease in young birds will significantly affect the seasonal pattern of salmonellosis as observed in this and other studies.

Unlike the distribution for salmonellosis, approximately 20% of cases of trichomonosis in greenfinches and chaffinches in the south were recorded in immature birds, and although in the north the figures were lower (10% and 12% respectively), the differences between regions were not statistically significant. The relatively high prevalence of trichomonosis in immature birds highlights how variation in bird factors and pathogen factors can influence the demography of disease. Salmonellosis in garden birds primarily causes disease in adults (see above), but immature birds appear to be more susceptible to trichomonosis than to salmonellosis. The causal agent of trichomonosis, *T. gallinae*, does not persist for long in the environment, but it colonises the upper digestive tract of adult birds and is easily passed to young birds when the latter are being fed. Further spread to immature birds from food and water contaminated by adult birds can then occur after parental feeding ceases. In contrast, *S. Typhimurium* is excreted in the faeces, and although it can persist in the environment for a long time, the infective dose by the faecal-oral route appears to be relatively high. The ease of transmission of trichomonosis to young birds explains in part the greater effect this disease has had at a population level, especially in greenfinches, compared with salmonellosis. Greater

involvement of young birds after fledging also accounts for some of the seasonal differences between salmonellosis and trichomonosis.

No cases of *E. albertii* bacteraemia were recorded in immature greenfinches in either region or immature siskins in the south, but 20% of cases of *E. albertii* bacteraemia in siskins in the north were in immature birds. This difference between siskins in the north and south was statistically significant ($p=0.003$, two-tailed Fisher's exact test) and may reflect the greater use of gardens in the spring and summer by siskins in the north than in the south (Table 7.9). This prolonged use of garden feeding stations in the north could increase exposure of the young siskins to organisms shed in the faeces by adults, resulting in mortality in the young birds.

To summarise, as was the case for month of onset and sex of birds affected, variation was seen in the age of birds affected by different conditions, dependent on region and species of bird, and driven by the interplay of pathogen factors, host factors and context factors as discussed in 7.2 above.

7.7 The role of supplementary feeding

Some of the advantages and disadvantages of providing supplementary food to wild birds are discussed in Chapter 1. The transmission of many infectious diseases is density-dependent (Swinton et al., 2001; Roche et al., 2012), so repeatedly attracting large numbers of birds into a small feeding area could increase the force of infection and result in mortality incidents. When salmonellosis was first noted to be a problem in greenfinches and house sparrows in the UK in the mid-to-late 1960s, it was suggested that artificial feeding conditions had resulted in a build-up of pathogens on and around bird tables (Macdonald and Cornelius, 1969). In some of these outbreaks the provision of large quantities of peanuts had attracted flocks of 100-200 greenfinches and house sparrows to the feeding stations. Chaffinches were not involved in these mortality outbreaks, probably because they are predominantly ground feeders and were not

attracted to the peanuts (Newton 1967). Siskins next developed the habit of feeding on peanuts, resulting in large flocks in some gardens (Spencer and Gush, 1973), further enhanced by a move from peanuts to sunflower hearts and nyger seeds in the 1990s (Moss 2003). Also in the 1990s, there was a resurgence of disease in garden birds, including siskins, at feeding stations, and Pennycott et al. (1998) suggested that deaths from salmonellosis in greenfinches and from *E. albertii* (then referred to as *E. coli* O86) bacteraemia in siskins were the result of the congregation of large numbers of birds feeding on peanuts and other seeds provided by the public. Kirkwood (1998) demonstrated that the risk of incidents of infectious disease in garden birds was partly related to the quantity of food provided at garden feeding stations, due to the increased population density, and noted that an estimated 15,000 tons of peanuts were fed to garden birds in the UK every year. Pennycott et al. (2005a) highlighted the link between bird numbers and the degree of contamination of a feeding station with *S. Typhimurium*, and suggested that the numbers of certain species of bird may be more important in garden bird salmonellosis than overall bird numbers.

Trichomonosis of garden birds has already been discussed in detail in this chapter and in Chapter 6, and is another example of a disease that can be spread at feeding stations. Lawson et al. (2012b) highlighted the marked rise in the number of woodpigeons seen in gardens in recent years, together with increased numbers of greenfinches and chaffinches prior to 2005, and speculated that spill-over of *T. gallinae* from woodpigeons to finches may have occurred at shared feeding stations. Of seven species of bird whose attendance at bird feeding stations in the UK had increased by over 20% prior to 2005, three were columbiforms capable of carrying *T. gallinae* (collared dove, woodpigeon and feral pigeon) and two were finches (greenfinch and chaffinch) susceptible to trichomonosis (Chamberlain et al., 2005). In recent years there has been a trend for feeding wild birds throughout the year rather than just in winter (Toms and Sterry, 2008), and such a change could increase the likelihood of inter- and intra-species spread. In particular, the provision of supplementary feed in the summer months, when adults are feeding young birds, could exacerbate the transmission of a pathogen such as

T. gallinae. Although this organism does not persist for long in the environment, it can be readily transmitted orally between adults at feeding stations and directly or indirectly from adults to young susceptible birds.

Changes to the type of food offered to garden birds have further expanded the range of bird species visiting garden feeders, potentially influencing the dynamics of disease. Toms (2011) described a “*meteoric increase in garden use*” by goldfinches since the mid-1980s, partly due to the provision of nyger seed and sunflower hearts at bird feeding stations. Offering nyger seed at bird feeders is also thought to be partly responsible for the 15-fold increase in the use of gardens by lesser redpolls since 2007 (BTO 2013b). Unfortunately, both species are also susceptible to salmonellosis and trichomonosis.

The most recent wild bird casualty of trichomonosis appears to be the turtle dove, a summer migrant whose UK population has declined by over 90% in recent years (Stockdale et al., 2015). These authors recovered *T. gallinae* from all 25 turtle doves sampled from three farms in East Anglia, England, and lesions typical of trichomonosis were found in the oropharynx of two nestlings and an adult turtle dove found dead. Concern was expressed that the disease may have arisen because of the practice of deliberately providing piles of grain on farms for turtle doves to feed on, to replace the natural wild seeds that are no longer available due to changes in arable farming methods. The resulting combination of high bird density at a small number of sites would increase inter- and intra-species contact rates, thus enhancing the transfer of pathogens. In these circumstances, Stockdale et al. (2015) cautioned that trichomonosis could significantly adversely affect the population size of this already-endangered bird. If turtle doves started to make greater use of bird feeders in gardens, as has happened with other species of pigeons and doves, the effects of trichomonosis could be even more devastating.

Another disease that can be acquired at bird feeding stations is infection with *Mycoplasma gallisepticum*, which has caused conjunctivitis and death in large numbers

of house finches (*Carpodacus mexicanus*) in the eastern USA (Hartup et al., 1998). These authors found that disease was significantly associated with the use of platform, tube or hopper bird feeders but not feeding from the ground. Tube feeders in particular were identified as causing increased pathogen transmission due to limited perch space and greater crowding potential, but the use of platform feeders provided more space and less bird-to-bird contact. House finches are not native to the eastern USA, but birds captured in the western USA were released in New York in the 1940s and in recent years many have started to congregate at back-yard feeders (Fischer et al. 1997). Finch mycoplasmosis is, therefore, another example of an infectious disease that can spread rapidly when a novel susceptible host species is attracted to bird feeding stations in large numbers.

Yet another recent “new” disease of garden birds that may be spread at feeding stations is avian pox in great tits. This condition was first confirmed in the UK in 2006, in the south of England (Lawson et al., 2012a). Since then, pox in great tits has spread further north and west, and Lawson et al. (2012a) commented that the congregation of birds at feeding stations could increase the transmission of poxvirus, especially if young susceptible birds were present when supplementary feeding was provided in the summer months.

Attracting large numbers of birds of many different species to garden feeding stations by the provision of supplementary feeding throughout the year may, therefore, contribute to the maintenance of endemic diseases such as *E. albertii* bacteraemia and salmonellosis, and to the appearance of new/emerging diseases such as mycoplasmosis and trichomonosis in finches and avian pox in great tits. Although density-dependent transmission is only one part of the complex net of inter-related factors that drive these diseases, nevertheless it is an area that human behaviour can directly influence. Not only does the provision of supplementary feed increase the number of birds at one location, it can also attract new species that may be more susceptible to infectious diseases or increase inter-species transfer of pathogens. There is a risk that disease may become

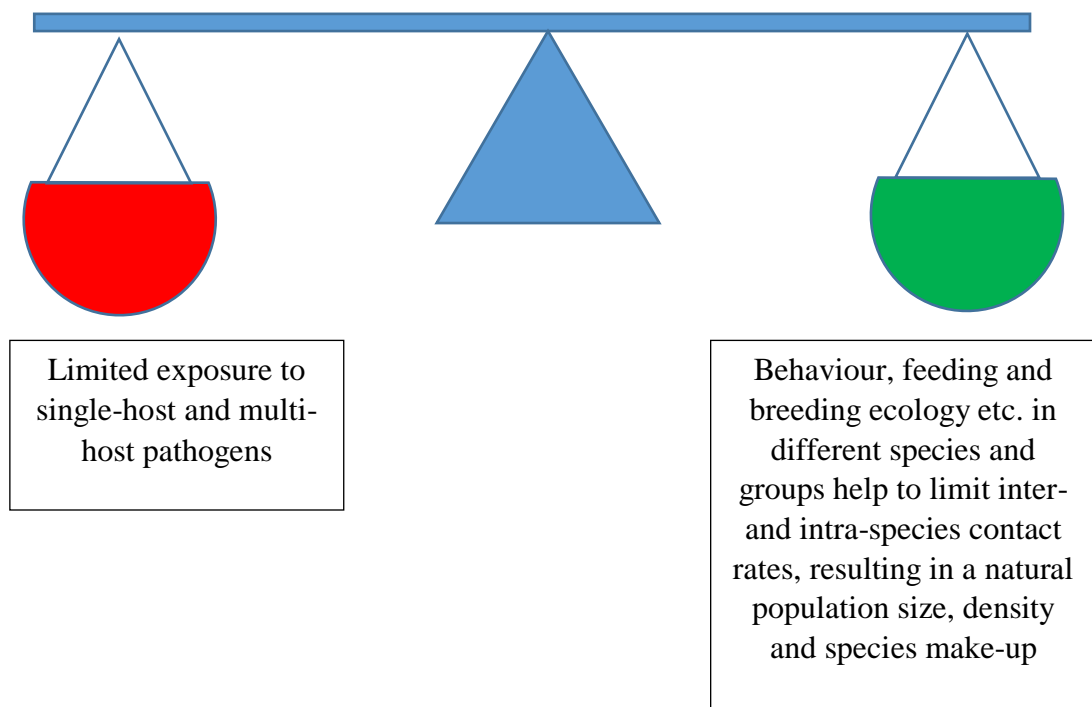
significant in less common species attracted to wild bird feeding stations, and cases of trichomonosis have already been recorded in bullfinches, reed buntings and yellowhammers, three of the priority species on the UK Biodiversity Action Plan (BAP) (http://jncc.defra.gov.uk/PDF/UKBAP_Species-HabitatsReview-2007.pdf). If turtle doves started to use garden feeders in England, the same fate could befall this BAP priority species.

The balance between garden birds and pathogens under “natural” conditions is depicted in Figure 7.13. Under such conditions, the feeding and breeding behaviours of different species of birds result in a “natural” population as regards its size, density and make-up, restricting inter- and intra-species contact rates and limiting exposure to single-host and multi-host pathogens. On occasion, additional factors such as adverse weather, concurrent disease, poor nutrition or elevated testosterone levels may “tip the scales” in favour of infectious disease in individual birds (Figure 7.14), but the balance should then return to that depicted in Figure 7.13. However, in the artificial situation of repeatedly attracting large numbers of birds of different species to relatively small feeding areas, there are unnaturally high inter- and intra-species contact rates and elevated exposure to pathogens that can infect multiple species, and the end result may be multiple deaths of a prolonged and significant nature (Figure 7.15).

When discussing the evolution and ecology of UK finches, Newton (1967) noted that, in order to exist in the same ecological niche, different species of animals or birds had evolved with different feeding and breeding strategies, most marked at times of the year when food availability was limited. Unfortunately, increased use of garden feeders may be breaking down these evolutionary safeguards. Cunningham et al. (2012), outlining some of the factors that can result in the emergence of new diseases in wildlife, stated that “*most emerging disease threats are caused by pathogens that capitalise upon the opportunities of exposure to transmit between species*”. They went on to say “*in most cases, one or more host species act as a reservoir for a pathogen that jumps species, with or without adaptation to the novel host, to infect a novel species that is at a twin*

disadvantage of being both susceptible and naïve”. The decision to provide large quantities of different types of supplementary food to wild birds throughout the year, periodically attracting novel species to the feeding stations is, therefore, not one to be taken lightly.

Figure 7.13: The balance between garden birds and pathogens under “natural” conditions



**Figure 7.14: The balance between garden birds and pathogens under “natural” conditions
– disease in individual birds**

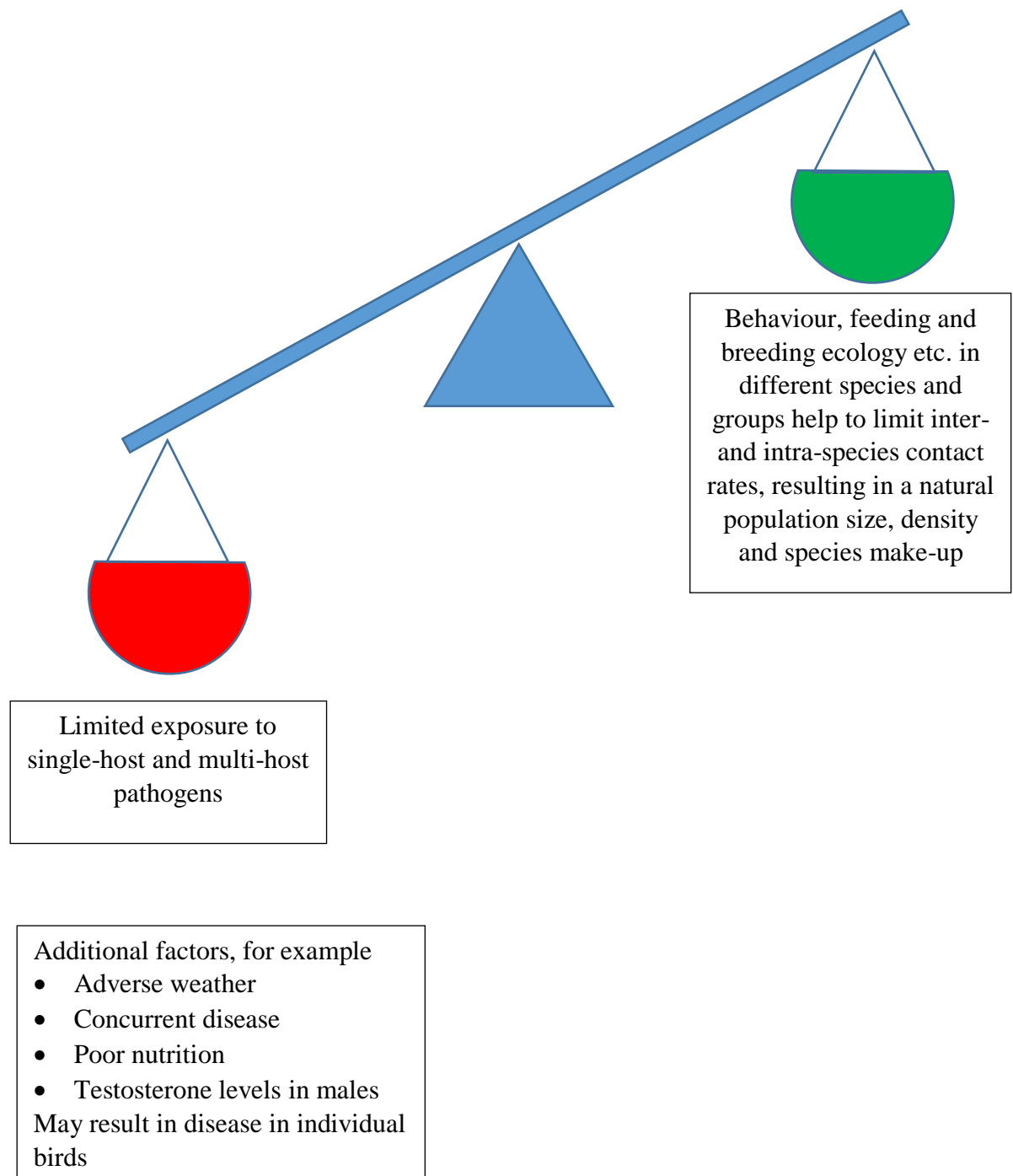
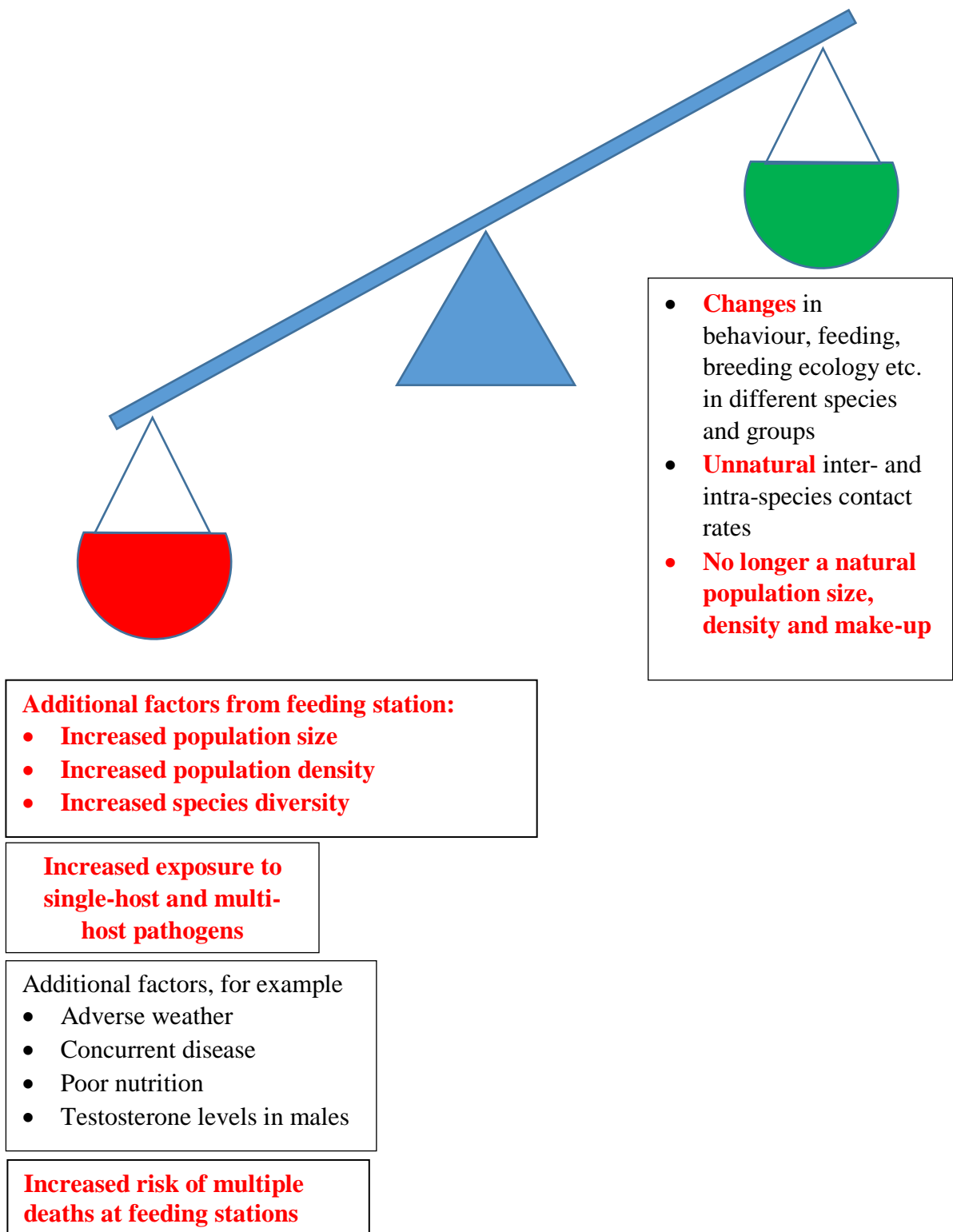


Figure 7.15: The balance between garden birds and pathogens – multiple deaths at some feeding stations



7.8 Measures to reduce losses from salmonellosis, *E. albertii* bacteraemia and trichomonosis

Salmonellosis, *E. albertii* bacteraemia and trichomonosis can become problems at garden bird feeding stations for a number of reasons, including: greater exposure to pathogens from contamination of the feeders, drinkers, baths and surrounding areas; increased intra-species contact; and increased inter-species contact. Different strategies can be employed to address these risks, albeit with varying degrees of likely success.

7.8.1 Reducing exposure to pathogens from contamination of the feeders, drinkers, baths and surrounding areas

This is the area of control on which most advice is usually given, focusing on good hygiene (see 1.2.2). Recommendations include: brushing bird tables daily to remove faeces and uneaten food; regular cleaning and disinfection of bird tables, feeders and baths using an appropriate disinfectant; cleaning under bird feeders; and moving the position of feeders on a regular basis. The use of multiple feeding sites may reduce the number of birds using each site, reducing contamination of the feeders and surrounding area. The design of the bird table or feeder can also influence the ease with which it can be contaminated by faeces and the feasibility of adequate cleansing and disinfection. Periodic complete breaks in feeding should be considered, partly to allow more thorough cleansing and disinfection, including natural antimicrobial actions such as desiccation and exposure to ultraviolet light, and partly to avoid the possibility of birds becoming too sedentary and dependent on the regular supply of artificially-provided food.

If unusually large numbers of sick or dead birds are found at bird feeding stations, feeding should be stopped to prevent further contamination of the feeding, bathing and roosting areas. Concern has been expressed by some householders that to cease feeding could cause problems if the birds have become dependent on the food supply, but if it has been the practice to deliberately incorporate non-feeding days or weeks into the

provisioning routine, then this should not be a problem. The view has also been expressed that if feeding at one site was stopped because of a disease outbreak, potentially infected birds would then move to other feeding stations, perhaps where hygiene etc. was poor. In reality, however, such movement would already be taking place, with the primary infected site acting as a disease “hub”, continually attracting and infecting healthy birds from other locations, some of which might die at the primary site but others would move and “seed” infection at other feeding stations. A cessation of feeding at all sites with evidence of disease is therefore crucial, and access to water for drinking or bathing should be managed in a similar fashion. Ideally, the cause of the mortality should be determined, for example by contacting the RSPB or Garden Wildlife Health (GWH), a collaborative group including ZSL, BTO and RSPB.

Improving the hygiene as outlined above may help to reduce exposure to pathogens at bird feeding stations, but the single biggest factor contributing to the size of the challenge dose is the number of birds visiting the feeding station on a regular basis, contaminating the site with faecal material, saliva and other upper digestive tract fluids, and, in the case of mycoplasmosis in house finches, oculo-nasal discharges. Hygiene alone will not counteract the pathogen load delivered by large numbers of birds repeatedly congregating in a small space. Bird numbers are heavily influenced by the quantity of food provided, and in the long term it may be beneficial to limit the quantity of food provided on any day or week.

Another option to be explored is to encourage greater availability of natural food sources in gardens (“wildlife-friendly gardens”), on waste ground, farmland and sporting estates. Gardeners can help to provide food for wild birds, by incorporating plants and trees that produce seeds and berries or that encourage invertebrates which form part of the natural diet of birds. Management of the garden by using fewer chemicals and delaying the removal of weeds or cultivated plants with seed-heads in the autumn will also help to provide food for birds. A wide range of “wild bird crops” is available for use on a larger scale (RSPB 2010), including different mixtures of linseed,

millet, kale, rape, quinoa, mustard, clover and cereals (oats and barley). The crops can be established in the spring to provide food for the wild birds in the winter and following spring, and financial support for farmers and sporting estates may be available as part of different rural development and land stewardship schemes. A similar approach may provide supplementary food for turtle doves in England in the summer months, by encouraging the growth of seed-producing plants such as fumitory, black medick, red and white clover, common vetch and birdsfoot trefoil, in gardens and on farmland (Anon 2015). Using natural food sources delivered in a natural fashion in large feeding areas should help to reduce some of the issues of contamination at small, heavily-provisioned, garden feeding stations. Also, by attracting insects, wild bird crops should also help to provide a range of invertebrates that will contribute to the diet of the birds. Nevertheless, Hayhow et al. (2015), reviewing the impact of agri-environment schemes on farmland bird populations, cautioned that although some schemes had benefited target species, other management options had resulted in only small positive effects or even negative effects. Further revisions to the management options and greater monitoring of results were recommended.

7.8.2 Reducing intra-species contact

As has already been noted, the transmission of many pathogens is density-dependent. Reducing the level of contact between susceptible and infectious birds of the same species will, therefore, reduce the likelihood of salmonellosis, *E. albertii* bacteraemia and trichomonosis at feeding stations. Measures to reduce bird numbers at each feeding station by using several feeders, by limiting the quantity of food provided, and by offering alternative “natural” food have already been discussed. Choice of feeder type and design can also influence intra-species contact rates. In addition, reducing intra-species contacts between susceptible and infectious birds can be achieved by restricting the time of year at which supplementary feeding is provided. Feeding during the summer months can facilitate the indirect transfer of pathogens from the feeding site to susceptible chicks in the nest by their parents. This is especially likely in adult greenfinches, which store food in their crop and then regurgitate it to their chicks,

enhancing the transmission of *T. gallinae*. Summer feeding can also increase the direct transfer of pathogens to young naïve fledglings that have left the nest and are congregating around potentially contaminated feeders and birdbaths. Such birds are then in direct and indirect contact with birds of the same species other than their parents, increasing the likelihood of disease transmission at feeding stations. The advice to feed throughout the year should be reviewed, and, in the event of a disease outbreak in the summer months, it is even more essential that feeding be stopped in order to reduce the spread of pathogens to nestlings and fledglings.

7.8.3 Reducing inter-species contact

As described in 7.2 and 7.7 above, contact between birds of different species can contribute to the emergence of new diseases and greater spread of existing diseases. In addition to the measures outlined above to reduce intra-species contact, consideration should be given to reducing inter-species contact by careful choice of food provided and the manner of provision. Whilst it is interesting to encourage unusual species of birds to visit garden feeders by offering different types of food, the downside can be greater opportunity for the spread of infectious diseases. If the introduction of a novel food source has the effect of attracting large numbers of unusual birds, it may be prudent to change the type of food or offer it in way that does not permit the new birds to feed. Perhaps a better alternative would be to provide natural food sources as described above, relying on the natural feeding strategies of different species to reduce inter-species contact rates.

Chapter 8

Diseases of UK blackbirds, thrushes, robins and starlings

8.1 Introduction

This chapter considers members of the families Turdidae and Sturnidae, omnivorous ground-foraging birds that eat a range of invertebrates and fruit. The family Turdidae includes the blackbird, song thrush, fieldfare (*Turdus pilaris*), redwing and robin, and also birds seen infrequently in gardens such as the mistle thrush, wheatear, ring ouzel, nightingale, redstart and various chats. The sole representative of the family Sturnidae likely to be seen in gardens in the UK is the starling. The Latin names of the UK birds discussed in this chapter are listed in Appendix I.

There are approximately 5.1 million breeding pairs of blackbirds in the UK, 1.2 million breeding pairs of song thrushes, 6.7 million breeding pairs of robins, and 1.9 million breeding pairs of starlings (Musgrove et al., 2013). Large numbers of fieldfares and redwings from Fennoscandia and northern Europe spend the winter in the UK or travel through the UK to reach France and Iberia, as do significant numbers of blackbirds and song thrushes and smaller numbers of robins. Musgrove et al. (2013) estimated UK winter populations of fieldfares and redwings to be around 700,000 birds for each species. In October and November, large numbers of starlings move into the UK from northern Europe and Fennoscandia, departing again in March and April (Feare 2002). The UK breeding population of starlings has fallen by about 50% in recent years, as has the number of migrants from the continent in winter (Feare 2002). The reasons for this decline are unclear but probably reflect changes in agricultural practice (Feare 2002). The starling and song thrush are red-listed as being of conservation concern in the UK (Hayhow et al., 2014).

8.2 Review of diseases found in UK blackbirds, thrushes, robins and starlings

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

8.2.1 Trauma

Trauma associated with human activities and domestic predators is one of the commonest causes of death recorded in blackbirds, song thrushes, robins and starlings that have been rung and the carcasses subsequently retrieved (Chamberlain and Main, 2002; Thomson 2002; Fennessy and Harper, 2002; Feare 2002). Such deaths include collisions with vehicles and buildings, cat predation, and deliberate killing of starlings for pest control. Mass mortality incidents have been reported in starlings that appeared to fly into vehicles, roads or water; Duff (2013) described deaths from trauma in at least 40 starlings that had been roosting near a busy road and then collided with vehicles, and Roberts (2014) reported a similar number of deaths in starlings that collided with the vehicle in which he was travelling. Barlow and Sparkes (2014) recounted a mass mortality incident in which 60 starlings died after flying on to a pavement and driveway, and multiple deaths were recorded in starlings that collided with a stationary ferry (Anon 2008b). Starlings frequently fly together in large flocks that rapidly change direction in unison (“murmurations”), sometimes close to ground level, and it is suggested that such incidents arise if the birds leading the flock make an error in flight direction. Other causes of trauma in starlings were highlighted by Feare (1984) and included aircraft strikes and birds falling down chimneys.

Lawson et al. (2015b) described 12 incidents of drowning in starlings, in ten of which at least ten birds were found dead. The birds drowned in garden ponds, swimming pools, a well and a bucket, and most often involved recently-fledged juvenile birds in May or June. A combination of strong flocking behaviour and inexperience of water was thought to be the underlying cause of the mass drowning incidents. Two incidents were also described in blackbirds, each involving a single bird (Lawson et al., 2015b).

Among the causes of trauma in robins listed by Mead (1984) was death in mouse traps baited with cheese, although cat predation was the commonest cause cited.

8.2.2 Pasteurellosis

Birds that survive attacks by cats and rats may then succumb to secondary pasteurellosis, because *Pasteurella multocida* can be found in the oral cavity of healthy cats and rats. Macdonald et al. (1981) described secondary pasteurellosis in blackbirds and starlings that died in a wildlife hospital, and such deaths usually occur within 48 hours of admission (Stocker 2000).

8.2.3 Salmonellosis

Salmonellosis caused by *S. Typhimurium* is a well-recognised cause of death of finches, sparrows, dunnocks and tits (see Chapter 4). However, this organism does not appear to be a common cause of death in blackbirds, thrushes, robins or starlings, despite close contact between these birds and infected environments around bird feeders. Wilson and Macdonald (1967) reported finding *S. Typhimurium* in only 2 out of 540 starlings caught in 1963, but provided no further details. Goodchild and Tucker (1968) were unable to detect *Salmonella* spp. in blackbirds (40 carcasses and 37 cloacal swabs), song thrushes (28 carcasses, 13 cloacal swabs), robins (4 carcasses, 68 cloacal swabs) or starlings (10 carcasses, 30 cloacal swabs). Examination of 41 samples of faeces from blackbirds, 14 from song thrushes, 13 from robins and 40 from starlings, all collected from birds caught at sewage treatment works, failed to recover any *Salmonella* spp. (Plant 1978). Discussing the isolation of *S. Typhimurium* from wild bird carcasses in Scotland between 1995 and 2003, Pennycott* et al. (2006) noted that *Salmonella* spp. were not isolated from 22 blackbirds, 8 song thrushes, 5 robins or 49 starlings. Looking at a similar dataset for England between 1993 and 2003, Lawson et al. (2010) did not detect *Salmonella* spp. in 66 blackbirds, 86 song thrushes, 8 robins or 16 starlings. Similar findings have been made elsewhere, for example in Norway, where salmonellosis is a problem in finches, the organism was not detected in 26 blackbirds or 11 robins found dead (Refsum et al., 2003). *S. Typhimurium* DT56v was isolated from faecal samples from two blackbirds and a starling feeding at a site where deaths from salmonellosis in finches and house sparrows were occurring (Pennycott* et al., 2002a) but no deaths from salmonellosis in blackbirds or starlings were recorded, and Hughes et

al. (2008) described the isolation of *S. Typhimurium* DT41 from the faeces of two healthy starlings. Higher recovery rates have been found in birds feeding on livestock units, for example Jones et al. (1983) isolated *S. Saint-Paul* from 20 out of 83 starlings caught on a dairy farm in England during an outbreak of salmonellosis in cattle, and Morishita et al. (1999) recovered *Salmonella* spp. from cloacal swabs from 62/868 clinically healthy starlings caught on or near livestock farms in Ohio, USA. In contrast, Gaukler et al. (2009) isolated *Salmonella* spp. from only 3 out of 436 starlings caught on a cattle feedlot in Kansas, USA, two of the isolates being identified as *S. Cholerae-suis*, a pathogen of pigs. Carlson et al. (2011) recovered *Salmonella* spp. from the intestinal contents of 2 out of 81 starlings caught at cattle farms in Texas, USA— as in the case described by Jones et al. (1983), the isolates were both *S. Saint-Paul*, but on this occasion this serotype was not recovered from cattle on the site. *S. Pullorum* and *S. Gallinarum* are two poultry-adapted serotypes that were common in poultry flocks in the UK prior to the 1970s and occasionally spilled over into wild birds including blackbirds (Wilson and Macdonald, 1967), but without causing overt disease. Overall, it appears that most serotypes of *S. enterica* do not cause mortality in blackbirds, thrushes, robins or starlings, although these birds may act as passive carriers if feeding in an infected environment.

Nevertheless, one serotype of *S. enterica*, *Salmonella enterica* serovar Hessarek (*S. Hessarek*) has caused deaths in blackbirds, song thrushes and starlings, sometimes in large numbers. Macdonald et al. (1968) described two outbreaks in garden birds in Scotland, mostly blackbirds but also starlings and house sparrows. Affected blackbirds were emaciated, had enlarged livers and spleens, and one had multiple abscesses in the breast muscles. This serotype was also recovered from 15 starlings and a house sparrow in Israel in 1976 (Singer et al., 1977) as part of an investigation into the causes of death of starlings at a large roost. As in the blackbirds in Scotland, affected starlings had swollen livers and spleens, but no necrotic foci or ingluvitis as seen in salmonellosis in finches. In Catalonia in north-east Spain, approximately 1000 migrating song thrushes died from *S. Hessarek* septicaemia in 2009, with smaller numbers found in 2010

(Velarde et al., 2012). Splenomegaly was the commonest gross postmortem finding, but hepatomegaly, sometimes with fine foci of necrosis, perihepatitis, necrosis of the pectoral muscles and multifocal encephalitis were also described in some birds. *S. Hessarek* appears to play a similar role in blackbirds, thrushes and starlings as *S. Typhimurium* does in finches and sparrows.

8.2.4 Trichomonosis

Trichomonosis in finches, buntings, sparrows, dunnocks and tits was reviewed in Chapter 6. The condition has also been seen occasionally in blackbirds, as a spill-over of disease from finches (Anon 2010b). One affected bird had a severe caseous necrotic oesophagitis that extended into the tissues surrounding the cervical vertebrae, and torticollis was observed in the bird prior to death (Anon 2010b).

8.2.5 Infection with *Chlamydia (Chlamydophila) psittaci*

Chlamydia (Chlamydophila) psittaci is a significant pathogen of birds that can also cause disease in humans (Beckmann et al., 2014a). From a wild bird perspective, *C. psittaci* most often causes disease in wild pigeons and doves (Gough and Bevan, 1983; Sharples and Baines, 2009), but disease has also been found in robins in the UK. Multiple deaths in robins, dunnocks and tits were reported from a garden in Cornwall, and two robins submitted for necropsy both had swollen livers with fibrin on the surface. *C. psittaci* was subsequently demonstrated in both birds by ELISA and in cell culture (Simpson and Bevan, 1989). Another case in a robin was described by Pennycott* et al. (2009) in which the liver and spleen were enlarged and liver necrosis and airsacculitis were present, and splenomegaly and hepatomegaly were described by Colvile et al. (2012) in three robins. Further genotyping of *C. psittaci* from three robins demonstrated Genotype A, which is also the commonest genotype identified in humans (Beckmann et al., 2014a). Although there is now good evidence that *C. psittaci* causes disease in robins, its significance in blackbirds, thrushes and starlings has not been established, partly because routine testing for this organism is seldom carried out in the UK due to financial constraints. Sharples and Baines (2009) reported negative results in three

blackbirds tested by PCR, and a larger study from Sweden (Olsen et al., 1998) detected *C. psittaci* by PCR in the faeces of only one out of 36 healthy blackbirds, song thrushes and redwings tested.

8.2.6 Infection with *Mycobacterium avium*

Mycobacterium avium, part of the *M. avium-intracellulare* complex, includes two subspecies, *M. a. avium* (the cause of avian tuberculosis), and *M. a. paratuberculosis* (the cause of Johne's disease in ruminants and possibly linked to Crohn's disease in humans). Before poultry keeping became intensified, avian tuberculosis frequently caused the death of adult chickens, and between 1913 and 1933 was the commonest diagnosis made in chickens submitted for postmortem examination in Scotland (Wilson 1960). Even by the early 1940s, 8-10% of deaths in adult chickens were diagnosed as cases of avian tuberculosis (Wilson 1960). During that era, avian tuberculosis was also seen in wild birds feeding alongside infected poultry flocks, for example Hignett and Mackenzie (1940) found 4.8% of starlings shot near an infected poultry flock had lesions of avian tuberculosis in the liver and/or spleen, and believed the true prevalence in starlings to be higher. In recent years, there has also been interest in the role that starlings may play in Johne's disease of ruminants, a chronic enteritis of worldwide importance caused by *M. a. paratuberculosis*. Evidence that this organism could be found in wild birds was presented by Beard et al. (2001), who cultured *M. a. paratuberculosis* from the tissues of 36/60 crows, 3/54 rooks and 1/38 jackdaws killed on four farms in Scotland with histories of Johne's disease in cattle or sheep. Starlings were not included in the Scottish study, but Corn et al. (2005) detected *M. a. paratuberculosis* in the tissues of 7/40 healthy starlings caught on cattle farms in the USA with histories of Johne's disease. Starlings may therefore play a role in introducing or maintaining the organism causing Johne's disease in ruminants. It has been suggested that starlings play a similar role in spreading the viral disease transmissible gastro-enteritis (TGE) in pigs (Anon 1999).

8.2.7 Avian pox and other skin conditions

Keymer and Blackmore (1964) commented that skin disorders appeared to be relatively common in robins, blackbirds and starlings, and appealed for field observations and specimens to be sent to them to permit further investigations. They subsequently reported that small wart-like lesions on the head of starlings and the wing of blackbirds were caused by avipoxvirus infection, and that mites such as *Microlichus avus* could cause feather loss in starlings (Blackmore and Keymer, 1969). These authors received multiple field reports of feather loss from the head of blackbirds, robins and starlings but limited material was submitted to determine the cause(s). Two robins had yellow or white scabbing of the head (and breast of one bird) and associated feather loss, and histopathology demonstrated hyperkeratosis of the skin and infiltration of the keratin and feather follicles by fungal hyphae. No pathogenic fungi were isolated, but the fungal elements seen on histopathology were considered significant. Similar lesions in robins were described by Soper and Hosking (1961) and Mead (1984). Despite numerous reports of feather loss in blackbirds, only one affected blackbird was received and examined by Blackmore and Keymer (1969), in which a heavy louse infestation was diagnosed. This may have been atypical because most reports had described feather loss from the head but the submitted carcass had feather loss from the neck, shoulders and under both wings. Similarly, although feather loss from the head of 23 starlings was reported to Blackmore and Keymer (1969), no material was submitted for further investigation. The cause of feather loss from the head of blackbirds and starlings must therefore remain speculative.

Feather loss around the eyes of a robin, arising from conjunctivitis caused by a nematode infestation of the conjunctival sac, is described in 8.2.11 below.

8.2.8 Ticks and tick-borne spirochaetes

Several studies have highlighted that blackbirds and thrushes are among the passerines most likely to be infested with ticks and to play a role in the epidemiology of tick-borne diseases. Pietzsch et al. (2008) recovered the nymph or adult stages of *Ixodes* sp. ticks

from blackbirds and song thrushes captured in the UK for ringing purposes, and James et al. (2011) identified *I. ricinus* larvae or nymphs in 18 out of 19 (95%) blackbirds and in both song thrushes caught in Scotland. The mean numbers of larval ticks per bird were also highest in blackbirds and song thrushes (James et al., 2011), with over 50 ticks present on one blackbird. These authors also found 45% of robins to be carrying larval *I. ricinus*, although the mean burden per bird was much lower than in blackbirds or thrushes. Similar results were reported from France (Marsot et al., 2012), who noted that blackbirds, song thrushes, robins and wrens (*Troglodytes troglodytes*) contributed most ticks to their study population, and from Switzerland (Humair et al., 1993) who found over 80% of blackbirds, song thrushes and robins were carrying *I. ricinus* larvae or ticks. James et al. (2011) demonstrated that a high proportion of the *I. ricinus* nymphs from blackbirds and song thrushes were positive by PCR for the spirochaete *Borrelia burgdorferi* sensu lato, and genotyping of 10 PCR-positives showed the spirochaetes to be *B. garinii*, one of the genotypes of *Borrelia* causing Lyme disease in humans. None of the nymphs collected from robins were PCR-positive. It appears, therefore, that blackbirds and thrushes could play a role in the epidemiology of Lyme disease in humans.

External parasites including ticks are also found on starlings, and Feare (1984) noted that starlings in the UK can carry three different species of tick: *I. ricinus* (the commonest), *I. arboricola*, and *Haemaphysalis punctata*. Other ectoparasites described in starlings by Feare (1984) included the hen flea *Ceratophyllus gallinae*, hippoboscids (louse flies), mites such as *Dermanyssus gallinae* and *Microlichus avus*, and several species of louse. Ticks, fleas, louse flies and lice have also been found in robins (Mead 1984).

8.2.9 Haematozoa and microfilaria

Haemoparasites of the genera *Plasmodium* (“avian malaria”) and *Haemoproteus* have been detected in wild birds of the family Turdidae in the UK (Anon 2013b), and Feare (1984) and Mead (1984) noted that UK starlings and robins can be infected by

Haemoproteus and *Leucocytozoon*. A checklist of species of *Haemoproteus* and *Leucocytozoon* found in birds (including blackbirds, thrushes, robins and starlings) was produced by Bennett et al. (1994). However it is unlikely that infected birds suffer any adverse effects (Bennett et al., 1993).

Microfilaria are the larval stages of filarioid nematodes of the family Onchocercidae that are released into the blood and/or subcutaneous tissues. The adult worms are long (1-5 cm) but thin and difficult to find because they are often in cryptic locations such as the connective tissues under the skin, around the trachea and oesophagus, or in the body cavity or heart; however microfilaria are readily detected if blood smears are examined (Bartlett 2008). When examining birds that had died during cold weather in 1963, Ash and Sharpe (1964) took the opportunity to examine blood smears from 48 birds (including 35 blackbirds and thrushes) for the presence of microfilaria. Twenty of the blackbirds and thrushes (57%) were positive for microfilaria, but none of the samples from other species were positive. It was not possible to identify the microfilaria, but Bartlett (2008) noted that filarioid nematodes of the genus *Eufilaria* are found in blackbirds. Adult *Eufilaria* spp. worms are found in the subcutaneous tissues around the trachea, oesophagus and crop, under the skin of the head and neck, and less commonly under the skin of the legs and groin (Bartlett 2008). They are spread by midges of the family Ceratopogonidae and usually are not believed to be pathogenic. Nevertheless, Simpson et al. (2014) described cardiomyopathy in a blackbird, in which adult filarioid nematodes up to 20 mm in length were located near the hock joints. Multiple foci of necrosis and granulomata were apparent in the myocardium, and microfilaria were noted in the capillaries of several organs including heart, lung and brain. The significance of the microfilaria, provisionally identified as those of *Eufilaria delicata*, was uncertain.

8.2.10 Internal parasites – isosporoid coccidia

The term “isosporoid coccidia” is used to describe two genera of coccidia, *Isospora* and *Atoxoplasma*, that produce faecal oocysts which, when sporulated, have two sporocysts (Schrenzel et al., 2005). Until recently, it was believed that *Isospora* spp. completed

their entire life cycle in the epithelium of the intestine, and *Atoxoplasma* spp. produced merozoites in blood or tissues. However, molecular techniques have cast doubts on these differences (Schrenzel et al., 2005), hence the preferred description as “isopsporoid coccidia”. Keymer et al. (1962) considered *Isospora* sp. to be the cause of death of two starlings, and detected *Isospora* sp. as incidental findings in other starlings, song thrushes and a blackbird. These authors cited other references to deaths from *Isospora* spp. in a starling, blackbird and song thrush, but unfortunately no further information was given as to the criteria on which these diagnoses were made. Mazgajski and Kedra (1998) demonstrated *Isospora* sp. in 67% of wild starling broods in Poland but found no evidence of harmful effects on the nestlings. However in a captive environment such as a zoo collection, the visceral form of coccidiosis has been shown to cause mortality in exotic starlings (Hafeez et al., 2014).

8.2.11 Internal parasites - helminths

Helminths are frequently recovered from the digestive tracts of blackbirds and thrushes. Ash and Sharpe (1964) reported that 61% of the blackbirds they examined had the acanthocephalan parasite (thorny-headed worm) *Polymorphus* sp., 14% had the cestode (tapeworm) *Hymenolepis* sp., and 14% had the gall-bladder trematode (fluke) *Lyperosomum* sp. Mistle thrushes had the tapeworms *Hymenolepis* and *Dilepis*; song thrushes carried *Polymorphus*, *Hymenolepis*, the trematode *Brachylaemus fuscatus*, and the large roundworm *Porrocaecum ensicaudatum*; and *Polymorphus* was found in redwings. [The acanthocephalans identified as “*Polymorphus*” were probably *Plagiorhynchus* (subgenus *Prosthorynchus*) *cylindraceus*, commonly found in birds of the families Turdidae and Sturnidae and sometimes in hedgehogs (Skuballa et al., 2009; Beckmann et al., 2014b)]. These birds had died during cold weather and the parasite burdens were not believed to be the primary cause of death. An earlier study (Ash 1957) looked at birds found dead in cold spells in 1954 and 1956 and found some blackbirds and thrushes were carrying heavy parasitic burdens – most commonly hairworms (*Capillaria* spp.), roundworms (*P. ensicaudatum*), acanthocephalans (“*Polymorphus*”)

and unidentified cestodes and trematodes. These authors concluded that heavily parasitised birds may be among the first to die during adverse weather conditions.

As part of a survey of wild bird mortality, Jennings and Soulsby (1957) reported that the commonest internal parasites found in blackbirds, song thrushes and starlings were *Porrocaecum ensicaudatum*, *Prosthynchus transversus* (now considered synonymous with *Plagiorhynchus* [*Prosthynchus*] *cylindraceus*), *Dilepis undula*, and *Liperosomum longicauda*. Keymer et al. (1962) found *Dilepis undula* in 10 blackbirds, a song thrush and a starling, and also recovered *Heterakis gallinae* (synonymous with *H. gallinarum*) from a starling and *Trichostrongylus tenuis* from a robin. Similar to the conclusions of the earlier authors cited above, these parasitic burdens were not thought to be of primary significance.

A comprehensive summary of the parasites found in starlings in England was provided by Owen and Pemberton (1962), who also listed other UK host species including blackbirds and thrushes. The commonest trematode found in starlings was the gallbladder fluke *Platynosomum perfoliata*, and the only acanthocephalan detected was *Prosthynchus cylindraceus*, now referred to as *Plagiorhynchus* (*Prosthynchus*) *cylindraceus*. *Hymenolepis farciminosa* and *Paricterotaenia parina* were the commonest tapeworms found in juvenile and adult starlings, whereas *Dilepis undula* was mostly restricted to immature starlings. *Syngamus trachea* was said to be almost ubiquitous in immature starlings, and hairworms of the upper digestive tract (*Capillaria contorta*) and intestine (*C. exilis*) were also common. Different stages of the large roundworm *Porrocaecum ensicaudatum* were frequently seen, but birds seldom had more than one adult worm.

Tracheal nematodes have been described in blackbirds, thrushes and starlings. Campbell (1935) found the gapeworm *Syngamus trachea* in 20/111 starlings (18.0%), with up to 5 pairs of worms in one bird. He also found *S. trachea* (then referred to as *S. merulae*) in 5/32 song thrushes (15.6%), 1/11 redwings (9.0%) and 12/72 blackbirds (16.6%), with

up to 7 pairs of worms present. These findings were similar to results from other sources cited by this author. Immature birds tended to be infected more often than adults and usually had higher burdens, but no indication was given that the parasites were causing disease. Gapeworms were not identified in robins by Campbell (1935) but fewer than 10 robins were examined.

An unusual nematode infection of the eyes of a robin found dead in the UK was reported by Beckmann et al. (2014b). Although the bird probably died from cat predation, the eyelids on one side were greatly swollen and there was extensive feather loss around the eye. Many nematodes 15-20mm in length and 0.5mm wide, subsequently identified as *Aprocta cylindrica*, were found in yellow gelatinous fluid under the skin around the eye. Secondary fungal infection of the damaged tissues was also present, and approximately 20 thorny-headed worms identified as *Plagiorhynchus cylindraceus* were found in the intestine. *Aprocta cylindrica* has been found in robins in central and eastern Europe, but this was the first confirmed recovery from a bird in western Europe, possibly a passage migrant. The intermediate host is believed to be a locust. Another parasite of robins is the skin fluke *Collyriclum faba*, which produces cysts under the skin of the vent, legs, and chest, and also around the eye and should therefore be considered in the differential diagnosis for *Aprocta cylindrica* infection. Literak and Sitko (1997b) demonstrated this parasite in 14/36 apparently healthy juvenile robins caught in Slovakia, but it has not yet been reported in the UK. The life cycle is thought to include a snail and a dragonfly larva as intermediate hosts (Literak and Sitko, 1997b).

The wide range of helminths (nematodes, cestodes, trematodes and acanthocephalans) found in blackbirds, thrushes, starlings and robins undoubtedly reflects the importance of earthworms, slugs, snails, insects and other invertebrates in the diets of these birds, which can act as intermediate or transport hosts for the parasites. Despite their common occurrence, sometimes in large numbers, with a few exceptions they do not appear to have a significant adverse effect on the host.

8.2.12 Encephalitis of unknown aetiology

Circling, ataxia, torticollis, head tremors and death have been reported in young starlings in Scotland since 1994 (Pennycott* et al., 2002b). Affected birds were usually observed in May or June, around the time they were due to fledge. Histopathology consistently demonstrated a non-suppurative encephalitis suggestive of a viral or protozoal infection, but no aetiological agent could be demonstrated by these authors. Cases were also seen in young house sparrows, but less frequently.

8.2.13 Adverse environmental conditions

Ground-foraging birds such as blackbirds and thrushes appear to be particularly susceptible to periods of prolonged cold weather. Ash (1957) reported that many blackbirds died in cold weather in 1954, and in 1956 “immense numbers” of mistle thrushes, fieldfares, song thrushes and redwings were found dead. Blackbirds were again affected in the cold winter of 1963 (Ash and Sharpe, 1964). Many of the carcasses examined were extremely emaciated. Prolonged severe weather in winter can also cause high mortality in starlings and robins, with deaths from starvation and hypothermia (Feare 1984; Mead 1984). In an unusual incident affecting migrating passerines in thick fog and rain off the east coast of England in October 2012, “thousands” of exhausted and disorientated birds landed on boats or in the sea (Anon 2012b). Most of the birds found dead in the water were redwings, fieldfares, blackbirds, song thrushes, robins, starlings and other smaller passerines.

8.2.14 Poisons and toxins

A tentative diagnosis of accidental mercury poisoning was made by Baker (1977) in 12 starlings found dead in a recently-sown field, and Feare (1984) highlighted the illegal use of mevinphos, alphachloralose and endrin to kill starlings in the 1970s. Yellow phosphorus poisoning in starlings and blackbirds was diagnosed by Jennings and Soulsby (1956) and by Jennings (1959), and dieldrin poisoning was recorded as the cause of death of two blackbirds by Keymer (1958). Twelve robins died from accidental

famphur poisoning; the product had been used as to treat cattle for warble flies, and the carcasses were found in the immediate vicinity of the cattle crush (Felton et al., 1981).

Suspected ethanol poisoning in blackbirds was reported by Duff et al. (2012) after 12 juvenile blackbirds were found dead at one location. Berries, most likely from rowan trees (*Sorbus* sp.), were present in the digestive tract of all the birds examined and some birds had traumatic injuries, but no other significant abnormalities were detected at necropsy. The liver of two birds and pooled gizzard contents were assayed and one liver had an ethanol content of 430 parts per million (ppm), a concentration considered to be toxic. A live blackbird from this site was seen to be behaving as if intoxicated by alcohol and subsequently made a full recovery. The same authors reported a similar episode in redwings that had been feeding on holly berries (*Ilex* sp.); birds were seen to fall out of the tree and died from trauma, and ethanol values of 1360 ppm were found in crop/gizzard contents, 465 ppm in intestinal contents, and 95 ppm in liver. Suspected ethanol toxicity has also been reported in cedar waxwings (*Bombycilla cedrorum*) feeding on berries of the hawthorn (*Crataegus* sp.) and Brazilian pepper tree (*Schinus* sp.) in the USA (Fitzgerald et al., 1990; Kinde et al., 2012). Typically in such incidents there was a history of consumption of large quantities of berries that were over-ripe or frost-damaged, or other factors had caused fermentation of the berries, increasing their ethanol levels.

Although botulism is mostly seen in waterfowl and seabirds, blackbirds, thrushes and starlings were also involved in the outbreak of botulism at St. James' Park, London, in the summer of 1969 (Keymer et al., 1972). No further details were provided.

8.2.15 Miscellaneous conditions and pathogens

Beak deformities

Pomeroy (1962) reported that beak abnormalities occurred most frequently in starlings, but were also seen in blackbirds, thrushes and robins. Possible causes included inherited defects, trauma and infectious agents.

Infection with Usutu virus

A major die-off of blackbirds occurred in Austria in the summer of 2001, caused by Usutu virus, a flavivirus related to West Nile virus and spread by mosquitoes (Weissenböck et al., 2002). Hepatomegaly, splenomegaly and mucoid enteritis were found at necropsy. Further cases were seen in blackbirds in Austria in the following years, with smaller numbers of great tits, thrushes and nuthatches (*Sitta europaea*) involved, and the disease was confirmed in blackbirds in Germany from 2011 (Horton et al., 2012). Antibodies to Usutu virus have been detected in some British birds (Buckley et al., 2003), but the specificity of the serology has been questioned (Horton et al., 2012). Targeted surveillance on tissues from 201 wild birds from the UK (including blackbirds, thrushes, robins and starlings) did not detect Usutu virus (Horton et al., 2012).

Mycotic pneumonia / airsacculitis

Aspergillosis was recorded in a blackbird (Jennings and Soulsby, 1957) and a starling (Jennings 1959) but no further details were provided.

Infection with Mycoplasma sturni

Rapidly growing mycoplasmas subsequently identified as *M. sturni* were recovered from a starling with conjunctivitis in the USA (Frasca et al., 1997) and from other asymptomatic wild birds of the families Sturnidae, Turdidae and Corvidae in the USA (cited by Pennycott* et al., 2005b). Screening of oropharyngeal and conjunctival swabs from wild birds in Scotland that had died from a range of conditions detected *M. sturni* in all three blackbirds tested, in all five starlings tested, and in 10 out of 23 corvids

tested (Pennycott* et al., 2005b). The pathological significance of *M. sturni* in birds with other conditions such as trauma, malnutrition, encephalitis and parasitism was unclear but doubtful.

Infection with Toxoplasma, Campylobacter

Samples of brain tissue from 133 starlings that had been culled from a city roost in England were screened for *Toxoplasma* spp. (a protozoan that can cause disease in birds, animals and humans) by passage through mice (Peach et al., 1989). Eleven out of 133 birds were positive, and the authors suggested that starlings could be a reservoir host for this organism. Another significant disease of humans is food poisoning caused by *Campylobacter* spp., especially *Campylobacter jejuni*. The possibility that starlings could be a direct or indirect source of infection was investigated by Colles et al. (2009). Starlings were caught by mist nets or were sampled while still in nest boxes at two sites in England. A high proportion of birds were found to be shedding *C. jejuni* (30.6%), *C. coli* was found in 0.6% of birds, and *C. lari* in 6.3%. However, molecular typing showed that the starling isolates were different from those found in humans or poultry, and the authors concluded that starlings did not pose a threat to humans or livestock in this respect.

Infection with Yersinia pseudotuberculosis

Yersinia pseudotuberculosis can cause focal lesions in liver and spleen that resemble avian tuberculosis, hence the name “pseudotuberculosis”. A wide range of birds can be affected, including birds of the families Turdidae and Sturnidae (Mair 1973).

8.3 Diseases of blackbirds, thrushes, robins and starlings examined at Ayr DSC 1994 to 2013 – results

8.3.1 Bird species, numbers and locations

Two hundred and seventeen blackbirds, thrushes, robins and starlings were examined between January 1994 and December 2013. Of the birds for which a location was given, 85% of blackbirds/thrushes were found in the south of Scotland, as were 76% of robins and 98% of starlings. A summary of the species examined and the conditions diagnosed can be found in Tables 8.1 – 8.4. The materials and methods are described in Chapter 2 and the diagnostic criteria used are listed in Appendix II.

Table 8.1: Blackbirds, thrushes, robins and starlings examined.

Species	Number examined
Blackbird	72
Song thrush	12
Redwing	5
Fieldfare	2
Robin	26
Starling	100
Total	217

Table 8.2: Conditions* diagnosed in blackbirds and thrushes (n=91).

Condition	Number of birds	% (95% confidence intervals)
Trauma	49	53.8 (43.1-64.2)
Significant helminthosis	10	11.0 (5.7-19.7)
Mycotic pneumonia / airsacculitis	9	9.9 (4.9-18.4)
Coccidiosis	8	8.8 (4.1-17.1)
Adverse environmental conditions	5	5.5 (2.0-12.9)
Miscellaneous conditions and pathogens:		All <5%
Cellulitis (secondary to trauma)	3	
<i>Pasteurella multocida</i> infection	2	
Feather/skin abnormality not otherwise specified	1	
Reproductive tract disorder	1	
Poisoning	1	
<i>Staphylococcus aureus</i> infection	1	
Trichomonosis (presumed or confirmed)	1	
No diagnosis	12	13.2 (7.3-22.3)
Incidental findings:		Not applicable
<i>S. Typhimurium</i> through selenite only	1	
Small numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Larval and nymphal ticks	1	
Helminth burdens not considered significant	Not recorded	
Oropharyngeal swabs screened for mycoplasmas – <i>M. sturni</i> isolated from three blackbirds	3	
Tissues screened for avian influenza virus – no positive results	26	
Tissues screened for West Nile virus – no positive results	31	

*Not all birds were examined for all conditions, and some birds had multiple conditions

Table 8.3: Conditions* diagnosed in robins (n=26).

Condition	Number of birds	% (95% confidence intervals)
Trauma	14	53.8 (33.7-72.9)
Adverse environmental conditions	4	15.4 (5.0-35.7)
Chlamydiosis / chlamydiasis	3	11.5 (3.0-31.3)
Miscellaneous conditions and pathogens:		All <5%
Abscess (<i>E. coli</i>)	1	
<i>Staphylococcus aureus</i> infection	1	
Feather/skin abnormality not otherwise specified	1	
Digestive tract condition not otherwise specified	1	
Trichomonosis (presumed or confirmed)	1	
No diagnosis	3	11.5 (3.0-31.3)
Incidental findings:		Not applicable
Small numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Helminth burdens not considered significant	Not recorded	
Oropharyngeal swabs screened for mycoplasmas – no positive results	1	
Tissues screened for avian influenza virus – no positive results	10	
Tissues screened for West Nile virus – no positive results	6	

*Not all birds were examined for all conditions, and some birds had multiple conditions.

Table 8.4: Conditions* diagnosed in starlings (n=100).

Condition	Number of birds	% (95% confidence intervals)
Trauma	23	23.0 (15.4-32.7)
Fledgling central nervous system disorder	57	57.0 (46.7-66.7)
Adverse environmental conditions	5	5.0 (1.9-11.8)
Miscellaneous conditions and pathogens:		All <5%
<i>Pasteurella multocida</i> infection	2	
Pox	2	
Developmental disorders (blindness)	2	
Significant helminthosis	1	
Central nervous system condition not otherwise specified (mycotic encephalitis)	1	
Mycotic pneumonia / airsacculitis	1	
<i>Yersinia pseudotuberculosis</i> infection	1	
Reproductive tract disorder	1	
No diagnosis	8	8.0 (3.8-15.6)
Incidental findings:		Not applicable
Small numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Helminth burdens not considered significant	Not recorded	
<i>M. sturni</i> isolated from oropharyngeal swabs	10	
Tissues screened for avian influenza virus – no positive results	14	
Tissues screened for West Nile virus – no positive results	38	

*Not all birds were examined for all conditions, and some birds had multiple conditions

8.3.2 Trauma and pasteurellosis

Trauma

Trauma was the commonest condition diagnosed in blackbirds (38/72, 52.8% of submissions [95% CI: 40.7-64.5]), song thrushes (8/12, 66.7% [95% CI: 35.4-88.7]), redwings (3/5, 60.0% [95% CI: 17.0-92.7]) and robins (14/26, 53.8% [95% CI: 33.7-72.9]), and was also frequently seen in starlings (23/100, 23.0% [95% CI: 15.4-32.7]). Secondary cellulitis was seen in some birds that survived the initial trauma. Where age and sex were recorded in blackbirds, 20 were immature birds, seven were adult females and five were adult males. The commonest causes identified in blackbirds were window collisions (9 birds), sparrowhawk predation (5 birds) and cat attacks (3 birds). Cats were

the commonest cause of trauma recorded in song thrushes (4 birds), and in robins the main causes were window collisions (3 birds), cats (3 birds) and sparrowhawks (2 birds). Trauma in starlings was most often the result of cat predation (5 birds) and crow or magpie attacks (5 birds, all nestlings/fledglings). Tissues from two blackbirds, found beside each other and with internal haemorrhage, were assayed for ethanol content with negative results.

Pasteurellosis

Secondary infection with *Pasteurella multocida* was diagnosed in two song thrushes and two starlings with traumatic lesions, all believed to be cat victims.

8.3.3 Infection with *Salmonella* Typhimurium

Liver and intestine were cultured from 56 blackbirds, 9 song thrushes, 2 redwings, 1 fieldfare, 20 robins and 70 starlings. *Salmonella* Typhimurium was isolated from one bird only (0.6% [95% CI: 0.0-4.0]), as an incidental finding through selenite enrichment from a blackbird that had died from trauma.

8.3.4 Infection with *Escherichia albertii*

Potentially-pathogenic *E. albertii* with the API-20E profile of 4144102 or 5144102 was isolated from 2/51 blackbirds (3.9% [95% CI: 0.7-14.6]), 1/20 robins (5.0% [95% CI: 0.3-26.9]) and 6/69 starlings (8.7% [95% CI: 3.6-18.6]) (Appendix VI). However none of the isolates was seropositive for O86:K61 and they were considered non-pathogenic. Sorbitol-fermenting strains of *E. albertii* with the API-20E profile of 4144502 or 5144502 were recovered from four blackbirds and 17 starlings with a range of conditions including aspergillosis, trauma and fledgling encephalitis, but the isolates were believed to be incidental findings. *E. albertii* is discussed more fully in Chapter 5.

8.3.5 Trichomonosis

Trichomonosis was confirmed in an adult blackbird from the north of Scotland ([Images 97-98](#)). The bird was thin and had thickening and necrosis of the upper oesophagus, with

yellow nodules visible from the serosa and causing a severe cellulitis. The lower oesophagus was greatly thickened and necrotic, resulting in almost complete obstruction of the oesophagus. Histopathology demonstrated a widespread necrotising oesophagitis associated with abundant protozoa consistent with trichomonads, and a PCR for *Trichomonas gallinae* was positive, with 100% sequence identity to the UK finch epidemic strain of *T. gallinae*. No other birds with trichomonosis were received from this site. Trichomonosis was also suspected in a robin with a caseous nodule in the oropharynx that died during an outbreak of trichomonosis in finches on the same site; this bird had in addition lesions typical of chlamydiosis, and a PCR for *C. psittaci* was positive (see 8.3.6 below).

8.3.6 Infection with *Chlamydia (Chlamydophila) psittaci*

Very few birds were screened for *C. psittaci* because of limited financial resources. Tissues from a blackbird with an enlarged liver and spleen of unknown cause, and from four fledgling starlings showing signs of a CNS disorder, were tested by PCR with negative results. Three out of six robins tested were positive by PCR – two birds that died during adverse weather conditions but with no gross lesions of chlamydiosis, and a robin that had an enlarged liver with an irregular pale necrotic area, an enlarged spleen, and a fibrinous airsacculitis ([Image 100](#)). This bird died during a large outbreak of trichomonosis in finches (Pennycott* et al., 2009) and also had a yellow caseous nodule on the hard palate ([Image 99](#)), and may therefore have had concurrent trichomonosis. Three robins in which no diagnosis was made were negative for *C. psittaci* by PCR: a bird in fat condition, a bird with an enlarged liver, and a bird with an enlarged liver and spleen and early pericarditis.

8.3.7 Avian pox and other skin conditions

Multiple nodules 2-4mm in diameter were found on the head of two adult starlings ([Images 101-104](#)). In one bird the nodules were around the eyes and at the commissures of the beak, and in the other bird the nodules were above and below the beak. The lesions were typical of avian pox, and the condition was confirmed in one bird by

histopathology and demonstration of poxvirus by electron microscopy. Feather loss and thickening/scabbing of the skin of the head and neck was seen in an adult blackbird and an adult robin. The lesions in the blackbird had been observed to develop over several months, and in addition to the head and neck, the leading edges and upper-sides of both wings were affected ([Images 105-109](#)). No external parasites were detected and no significant bacteria were isolated from the skin. KOH preparations demonstrated many fungal hyphae, and histopathology revealed marked hyperkeratosis and acanthosis, with vast numbers of thin filamentous septate fungal hyphae plus smaller numbers of large irregular non-septate hyphae. The fungi *Mucor* sp. and *Penicillium* sp. were cultured from the skin lesions, but it was unclear if they were the same fungi as detected on histopathology. Only the head and neck of the robin were affected ([Image 110](#)), and fluorescence under UV light was marked. No further tests were carried out but a fungal aetiology was again suspected.

8.3.8 Isosporoid coccidia

Coccidiosis caused or contributed to the deaths of seven immature blackbirds and an immature song thrush, all in the months July to September. Coccidiosis was not diagnosed in robins or starlings, although coccidial oocysts were frequently seen in starlings as an incidental finding.

Five of the seven blackbirds were being reared in a wildlife rehabilitation centre, and had been transferred to outdoor aviaries in the two weeks prior to death. Affected birds were submitted for necropsy in three different years, and similar losses had occurred in other years. Despite being in good body condition when transferred, the birds were all thin when found moribund or dead in the outside enclosures. Postmortem examinations revealed marked thickening of the duodenum and small intestine, and wet preparations demonstrated large or very large numbers of unsporulated coccidial oocysts ([Images 113-118](#)). Sporulation of oocysts from two affected birds confirmed the coccidia to be isosporoid, with two sporocysts ([Images 119-122](#)). Concurrent burdens of helminths including *Syngamus trachea* (gapeworms), tapeworms, hairworms and large

roundworms were frequently present. Histopathological examination of the intestine of three affected birds revealed a severe transmural lymphocytic enteritis, with heavy infiltration of lymphocytes into the mucosa and submucosa, extending through the muscularis to the serosa (Images 123-125, 131). Large numbers of coccidial stages (mostly macrogametes and microgametes, and some oocysts) were found in the epithelial cells of the villi and crypts and in the lamina propria (Images 126-130, 132-138). No significant abnormalities were detected in the liver of affected birds.

The remaining two immature blackbirds with coccidiosis were found dead in two different gardens. One had marked thickening of the intestine associated with large numbers of coccidial oocysts, hairworms and tapeworms, and histopathology demonstrated very similar findings to those seen in the birds that died at the rehabilitation centre. The other blackbird, in addition to large numbers of coccidial oocysts, had over 200 acanthocephala (thorny-headed worms) (Images 179-180), many small ticks and lice, and a fractured wing with granuloma formation.

One immature song thrush found dead in a garden had pink semi-fluid digesta distending the intestine, associated with very large numbers of coccidial oocysts (Images 111-112). Tapeworms, hairworms and gapeworms were also present, and a terminal *Staphylococcus aureus* bacteraemia was found. Further details about the coccidial oocysts can be found in Appendix III.

8.3.9 Helminths

Helminth burdens likely to have had a negative impact were found in eight immature blackbirds. Two birds were from a rehabilitation centre and had coccidiosis as described above, and in addition had 10-12 pairs of the gapeworm *S. trachea*. Another two birds that lost weight and died after being placed in outside aviaries at the rehabilitation centre 2-3 weeks earlier had no coccidial burdens but had significant mixed burdens of either gapeworms, tapeworms (Image 162), *Porrocaecum* sp. (large roundworms) (Images 142, 162) or thorny-headed worms (Image 181). Two birds that had not been in a

rehabilitation centre had large burdens of coccidia and either hairworms, tapeworms or thorny-headed worms, and a fledgling blackbird had two pairs of gapeworms associated with congested lungs and much bloody fluid in the trachea. Another thin immature blackbird had a grossly thickened oesophagus with many hairworms visible on microscopy, several gapeworms in the trachea, moderate numbers of coccidia and *Porrocaecum* sp., and many thorny-headed worms in the intestine. Histopathology showed marked roughening and focal erosion/ulceration of the mucosa of the oesophagus associated with hairworms, and a granulocytic pneumonia possibly caused by gapeworm larvae.

Parasite eggs provisionally identified as schistosome-like eggs contributed to the deaths of two adult blackbirds. One blackbird was in fair body condition, had an enlarged liver, moderate burdens of tapeworms, and marked thickening of the duodenum and small intestine. Histopathology revealed an extensive granulomatous enteritis and hepatitis associated with egg-like structures. Most of these structures had a well-defined thin eosinophilic outer envelope, with an underlying thicker cellular layer, sometimes separated from the outer envelope by a small space (Images 226-231, 234-235, 242-249). Contained within these layers was a central multicellular mass, separated from the outer layers by a clear space. In some eggs only one layer was apparent (Images 240-241, 250), and sometimes the multicellular core was immediately contiguous to the outer layer(s). The histopathological appearance of the eggs did not resemble eggs of nematodes or thorny-headed worms (Images 263-270) or coccidial oocysts (Image 271) but were similar in appearance to schistosome eggs seen in the small intestine of a mute swan (Images 260-262). They also resembled schistosome eggs found in human tissues (Images 232-233, 236-239). The eggs from the blackbird tissues were, therefore, provisionally identified as schistosome-like eggs, and the central multi-cellular mass within the outer shell was considered to be a miracidium.

Wet preparations from the intestinal mucosa and contents of this bird demonstrated large numbers of spherical or sub-spherical eggs, with a thin smooth outer membrane. The

contents of the eggs were dark and granular, and either filled the egg or had contracted, leaving a space between the inner mass and the outer membrane (Images 201-203, 205-209). They did not resemble the eggs of tapeworms, nematodes, thorny-headed worms or intestinal flukes encountered in other blackbirds in this study. They were, however, of a similar magnitude to those found in H&E sections of intestine and liver of this bird, and were considered to be the same organisms (i.e. schistosome-like), with the central mass representing a miracidium contained within a thin shell. Further supporting evidence that the eggs observed in wet preparations resembled schistosome eggs was acquired by comparing them with eggs of human schistosomes (Images 217-220) and from a mute swan (Image 37). Pathology associated with schistosome eggs, including granulomatous changes in the liver and intestine, has been described in humans (Gryseels et al., 2006) and in avians (Horak et al., 2015), and it appears that schistosome-like organisms can cause similar pathology in blackbirds.

Degenerating schistosome-like eggs were seen on histopathological examination of the liver of a second adult blackbird, which had enlargement and necrosis of the liver (Image 253) and spleen. Histopathology showed a chronic multifocal granulomatous hepatitis with central areas of necrosis, with occasional cross-sections of parasitic eggs in the blood vessels or bile ducts of the portal tracts (Images 254-258). Moderate numbers of internal parasites (tapeworms and roundworms) were seen grossly in the intestine but no wet preparations were examined from this bird. Schistosome-like eggs were, however, noted in the intestinal contents of an immature blackbird that developed cellulitis after a cat attack, in a thin adult blackbird for which no diagnosis was made, in a fat immature blackbird that died after colliding with a window, and in an adult bird that died from trichomonosis. No histopathology was carried out in these birds and the significance of the eggs was unclear.

Very similar eggs were recovered from the intestinal mucosa or contents of three dunnocks (Images 204, 210-211), a redwing (Images 215-216), a house sparrow (Images 212-214), a tawny owl (Images 221a and 221b) and a sparrowhawk (Images 222a -

222b), and the dimensions of those eggs and those from blackbirds are summarised in Table 8.5. As in one of the blackbirds, large concentrations of eggs were found in fragments of intestinal mucosa from two of the dunnocks (Image 204). Examination of previously frozen intestinal material from two dunnocks showed some eggs in which the contents appeared to be exiting a thinner outer shell that had split (Images 27-28), possibly corresponding to the thin outer eosinophilic membrane observed on the histopathological examination of blackbird tissues described above. In the case of the tawny owl and sparrowhawk, the eggs may have been pseudo-parasites released from the digestive tract of prey items such as small birds. No histopathology was carried out in these other species, but in one thin dunnock large numbers of these eggs were associated with very fluid intestinal contents.

In addition to the cases discussed above, helminths were detected in other blackbirds, redwings, song thrushes, robins and starlings, but were considered to be incidental findings except in one bird, an immature starling with eight pairs of gapeworms wrapped in a tight bundle and causing an obstruction of the trachea. Further details of the helminths and eggs described above can be found in Appendix III.

Table 8.5: Dimensions (and range) of schistosome-like eggs from intestinal contents or histopathology of intestine and liver.

Source of eggs	Number measured	Length and width of eggs: mean (range)
Wet preparations from intestine of 2 blackbirds	37	74 μ m (47-109) by 72 μ m (59-109)
Wet preparations from intestine of 3 dunnocks	110	75 μ m (54-105) by 68 μ m (53-97)
Wet preparations from intestine of 1 redwing	24	72 μ m (53-101) by 61 μ m (44-96)
Wet preparations from intestine of 1 house sparrow	38	75 μ m (61-110) by 68 μ m (52-99)
Wet preparations from intestine of 1 tawny owl	5	85 μ m (75-96) by 77 μ m (70-84)
Wet preparations from intestine of 1 sparrowhawk	6	89 μ m (79-99) by 76 μ m (64-84)
Intestinal histopathology from 1 blackbird – solid eggs	7	63 μ m (54-70) by 58 μ m (49-68)
Intestinal histopathology from 1 blackbird – hollow eggs	8	86 μ m (73-92) by 80 μ m (69-86)
Liver histopathology from 1 blackbird – solid eggs	10	62 μ m (48-73) by 55 μ m (44-65)
Liver histopathology from 1 blackbird – hollow eggs	15	74 μ m (63-91) by 67 μ m (55-74)

8.3.10 Disorders of the central nervous system

Clinical signs suggestive of a central nervous system (CNS) disorder were reported in sixty-six nestling or fledgling starlings received for necropsy in May and June ([Images 272-277](#)). Signs included torticollis, circling, ataxia and tremors of the head. Five of the birds had obvious head trauma and no further testing was carried out, although it is possible that other underlying brain pathology was present. Another bird, a nestling found under its nest, had a large area of brain necrosis grossly visible; histopathology subsequently demonstrated a large necrotic area in the cerebrum and cerebellum associated with degenerating granulocytes and numerous septate fungal hyphae, and a diagnosis of mycotic encephalitis was made. Of the remaining 60 birds, histopathology was carried out in 22 birds; no histopathological lesions were detected in three birds (recorded as “no diagnosis”) but evidence of a non-suppurative encephalitis (NSE) was

found in 19 birds and a diagnosis of “fledgling CNS disorder” was recorded. For financial reasons, histopathology was not carried out in the remaining 38 birds, and in the absence of obvious trauma a presumptive diagnosis of “fledgling CNS disorder” was recorded in these birds also. The histopathological lesions found in six affected starlings were described by Pennycott* et al. (2002b) and were typical of those found in all 19 birds. There was microgliosis, neuronophagia and perivascular cuffing by mixed mononuclear cells, mostly in the cerebrum and midbrain (Image 278), and in a few birds there was a severe vasculitis and malacia of the surrounding tissues. Histopathology was also carried out on spinal cord, cardiac muscle and skeletal muscle from four affected birds, but no significant abnormalities were detected. One bird with severe NSE that was examined subsequent to the 2002 publication was different in that clusters of structures consistent with protozoal stages were associated with some of the perivascular cuffs (Image 279). These bodies were basophilic, round to ovoid, and appeared to be arranged in a radial fashion within a capsule. Immunoperoxidase (IPX) staining for *Toxoplasma* spp. and *Neospora* spp. was carried out by AHVLA Lasswade on sections of brain from this bird with negative results, but no suitable antigen was available to permit IPX for *Sarcocystis* spp.

Pennycott* et al. (2002b) described attempted virus isolation in embryonated eggs, Vero cells and other cell lines, and in day-old mice. The only virus detected was a reovirus, isolated from two out of three birds; intestine of one bird, and intestine and pooled liver/brain of a second bird. In addition, tissues from 12 birds were screened by AHVLA for avian influenza virus and West Nile virus, but no positive results were received. Brain, oropharyngeal and conjunctival swabs from eight starlings with fledgling CNS disorder were tested for *Mycoplasma gallisepticum* and *M. synoviae* by PCR at Liverpool University, with negative results. The tissues were also cultured, and *Mycoplasma sturni* was isolated from the oropharynx or conjunctiva of all eight birds and from the brain of two birds.

Histopathology was carried out on the bursa of Fabricius of seven fledgling starlings, five with and two without NSE. No abnormalities of the bursa were found in three birds with NSE, but the other four birds (two with NSE, two without NSE) all had moderate or marked bursal atrophy. There was depletion of lymphocytes from the medulla and to a lesser extent the cortex of the follicles, some follicles were cystic, and others had large numbers of pale macrophages/reticular cells in the medulla. No viral inclusion bodies were noted in any of the bursae. The severity of the lesions suggested that some degree of immunosuppression was present. Intestine from five fledgling starlings showing signs of a CNS disorder were tested by PCR for pigeon circovirus as part of a different study; one sample was strongly positive, one was weakly positive and the other three were negative.

8.3.11 Mycotic pneumonia / airsacculitis

A diagnosis of mycotic pneumonia or airsacculitis was made in nine blackbirds, all immature, and in one adult starling. Most of the blackbirds were received in April to June, but single cases were seen in July and October. One brood of four nestlings aged approximately 10 days had been removed from the nest and taken to a wildlife rehabilitation centre. The birds showed respiratory distress the following morning and died, and postmortem examination showed multiple tiny yellow nodules in the lungs of all four birds ([Images 280-281](#)). Other cases had not been in care but were found dead in gardens and typically had multiple cream or yellow nodules in the lungs and sometimes on the airsacs ([Images 282-285](#)). Some birds also had large numbers of intestinal tapeworms and one had numerous intestinal flukes. *Aspergillus fumigatus* was consistently isolated from the lungs. One case of mycotic pneumonia was observed in a starling with a large nodule in the lung, but unlike the blackbirds this bird was adult and no fungi were isolated. However, histopathology subsequently revealed a granulomatous pneumonia associated with numerous septate branching hyphae typical of *Aspergillus* sp. In addition there were cross-sections of what appeared to be gapeworm larvae in the pneumonic areas, which may have predisposed to the mycotic pneumonia.

8.3.12 Adverse environmental conditions

Three redwings and two fieldfares died as a result of very cold weather in three different winters. Prolonged cold weather was believed to be the cause of death of two adult robins, and excessively wet weather was responsible for the death of two immature robins. Nestling starlings appeared to suffer from the opposite extreme, with deaths in five birds occurring during unusually hot weather, representative of larger scale mortality, in two different years. The birds were being reared in nests under roof tiles, and the only consistent significant abnormality detected at necropsy was pallor of the kidneys.

8.3.13 Miscellaneous conditions and pathogens

Digestive tract conditions not otherwise specified

The gizzard of a thin immature robin found dead on a farm was distended and impacted with fibrous material of unknown origin.

Reproductive tract disorders

A fat starling had a broken fully-shelled egg impacted in the oviduct, and egg yolk peritonitis caused the death of a blackbird.

Yersinia pseudotuberculosis and Staphylococcus aureus

Y. pseudotuberculosis was isolated from the tissues of a thin adult starling with an enlarged liver and spleen containing multiple necrotic foci (**Images 286-287**). Terminal *S. aureus* bacteraemia was present in an immature song thrush that died from coccidiosis, and heavy growths of *S. aureus* were recovered from the heart, liver and spleen of a robin with pericarditis, airsacculitis and hepatomegaly.

Cellulitis and abscesses

Three blackbirds that were believed to be cat victims had, in addition to traumatic injuries, plaques of inflammatory debris beneath the skin from which mixed bacteria

were isolated. Heavy pure growths of *E. coli* were recovered from an abscess under the skin of the neck of a robin.

Developmental abnormalities

Two nestling or fledgling starlings from the same brood had eye problems; one had grossly normal eyes but was clinically blind, and the other had a domed skull and no eyes (Images 288-289). Both birds were euthanased.

Poisoning

Bendiocarb poisoning was confirmed in an adult blackbird in an incident in which bread treated with bendiocarb was illegally used to poison gulls.

8.3.14 No diagnosis

No diagnosis was made in nine blackbirds and three song thrushes. Most were probably cases of undetected trauma or “orphaned” fledglings. Some blackbirds in very good condition with much food in the digestive tract had marked enlargement of the liver and/or spleen (Images 290-293), but no cause was found; a PCR for *C. psittaci* was negative in one bird, and a PCR for *Borrelia* spp. was negative in one bird. These birds may have been cases of undetected trauma. No diagnosis was reached in three robins, all in good body condition. One had an enlarged liver and one had an enlarged liver and spleen and early pericarditis, but no significant bacteria were isolated and a PCR for *C. psittaci* was negative for all three birds. No diagnosis was reached in eight starlings, mostly “abandoned” fledglings or fledglings with CNS signs but with no significant lesions on histopathology and no obvious evidence of trauma.

8.3.15 Incidental findings

Swabs (oropharyngeal, sometimes also conjunctival and brain) from four blackbirds, one song thrush, one robin and twelve starlings were screened by PCR for *M. gallisepticum* and *M. synoviae*, with negative results. The same swabs were cultured and *M. sturni* was isolated from three immature blackbirds and ten immature starlings. The blackbirds had

died from aspergillosis and the starlings had exhibited signs of a CNS disorder prior to death, and the significance of the findings was unclear. These results expanded on those reported by Pennycott* et al. (2005b).

Tissues from 50 birds were sent to AHVLA Weybridge to be screened for avian influenza viruses, but no positive results were reported. Similarly, tissues from 75 birds were sent to AHVLA to be screened for West Nile virus, but no positive results were received.

8.4 Discussion

8.4.1 General comments

Most of the carcasses originated from the south of Scotland and the results may not have been representative of conditions seen further north. Nevertheless, the findings will be of interest to wildlife rehabilitators and their veterinary advisers throughout Scotland. One of the most significant findings in this group of birds was the virtual absence of three infectious diseases that accounted for nearly three-quarters of deaths of finches, buntings, sparrows, dunnocks and tits, namely salmonellosis, *E. albertii* bacteraemia and trichomonosis (see Chapters 4-7). *S. Typhimurium* DT56v was an incidental finding in a blackbird that had died from trauma, all isolates of *E. albertii* that were serotyped were O86:K61 negative and considered non-pathogenic, and only two birds, a blackbird and a robin, died from suspected or confirmed trichomonosis. The differences between the two groups of garden birds are all the more remarkable when you consider that blackbirds, thrushes, robins and starlings frequently feed on the ground under or near feeding stations at which birds with these infectious diseases are present, and on some occasions share the actual feeding station. Similarly, the two groups of birds may share the same birdbaths, frequently soiled by faeces, for bathing or drinking. There can be little doubt that blackbirds, thrushes, robins and starlings will be exposed to these pathogens, yet evidence of disease is rare. Different groups of garden birds appear to be innately susceptible or resistant to different pathogens, but there is a danger that minor changes in the pathogens could overcome this natural resistance, resulting in new epidemics of disease. The absence of avian tuberculosis in starlings was also of interest, and probably reflected the substantial reduction in cases in the national poultry flock following intensification.

Rather than infectious disease, trauma was the commonest cause of death in blackbirds, thrushes and robins, accounting for over half of all submissions received. Where a cause was identified, sparrowhawk attack, cat predation and window collision were the commonest causes. Trauma was also commonly seen in starlings, and predation of

immature starlings by crows or magpies was observed or suspected in five birds. Prolonged cold weather resulted in the deaths of fieldfares and redwings, migrants from Fennoscandia and northern Europe that may already have been weakened by adverse conditions prior to their arrival in Scotland, and the same may also have been true for two adult robins that died during cold weather. It was interesting to note that this diagnosis was not made in any blackbirds during this surveillance period; Ash (1957) reported that in the cold spell in 1956, fieldfares, redwings and thrushes were affected but not blackbirds, and speculated that many blackbirds had moved away at the start of the cold weather. Skin conditions seen in blackbirds, robins and starlings in the current study were remarkably similar to those described by Blackmore and Keymer (1969) and need not be further discussed here.

This twenty-year study has increased awareness of *C. psittaci* infection in robins and the importance of coccidiosis, helminthosis and aspergillosis as causes of death of immature blackbirds, especially but not exclusively in rehabilitation centres. By far the commonest diagnosis in submitted starlings was “fledgling CNS disorder” but the aetiology remained elusive. These conditions are discussed more fully below, but again highlight differences between diseases seen in finches, sparrows, dunnocks and tits (Chapters 3-7) and blackbirds, thrushes, robins and starlings (this chapter). Similarly, carriage of *M. sturni* was detected in a high proportion of blackbirds and starlings tested, but not in finches or house sparrows.

8.4.2 Infection with *C. psittaci*

C. psittaci is an important pathogen that can spread from birds to humans, and in this study three robins were PCR-positive for *C. psittaci*. Two of the birds had died during very cold weather and one bird had oropharyngeal necrosis suggestive of concurrent trichomonosis in addition to lesions of chlamydiosis. Colvile et al. (2012) detected *C. psittaci* in three robins, one of which also had an extensive salpingitis associated with unidentified trematode eggs, and in a second bird immunohistochemical labelling suggested the organism was not associated with gross pathology. Beckmann et al.

(2014a) demonstrated *C. psittaci* in a nestling robin, one of a clutch of three found dead in the nest, in which the only significant postmortem abnormality was hepatic congestion. These findings suggest that some healthy robins carry this potentially zoonotic organism, sometimes resulting in pathology triggered by other infectious or non-infectious factors but on other occasions detected by PCR as an incidental finding in birds dying from other causes. Nevertheless, in the cases of chlamydiosis in robins reported by Simpson and Bevan (1989), no trigger factors were described and the source of the organism was believed to be collared doves. A more detailed structured study is required to establish the carriage and significance of *C. psittaci* in robins.

8.4.3 Isosporoid coccidiosis and significant helminthosis in blackbirds

Postmortem examinations of immature blackbirds dying in a rehabilitation centre revealed that intestinal coccidiosis and/or helminthosis were important causes of death, being diagnosed in birds in several years but suspected on other occasions when necropsies were not conducted. This is believed to be the first report of coccidiosis in blackbirds in a rehabilitation centre in the UK, but fatal coccidiosis has been reported to be a significant problem in young American robins (*Turdus migratorius*, more closely related to the European blackbird than the European robin) being reared in a wildlife rehabilitation centre in the USA (Xie and Nevis, 2012). In both Scotland and the USA, coccidiosis developed when the birds were transferred to large outdoor enclosures prior to release, where presumably they were faced with an overwhelming challenge of oocysts from the environment. Substantial thickening of the intestine was noted in the blackbirds dying in Scotland, and histopathological examination of cases from Scotland and the USA showed a severe lymphocytic enteritis similar to that observed in captive finches and warblers with coccidiosis in America (Middleton and Julian, 1983; Swayne et al., 1991). The inflammatory reaction was so severe that it resembled a neoplastic lymphoproliferative disease, but Swayne et al. (1991) concluded that the lymphocytic infiltration represented an exaggerated inflammatory or immunological response. Isosporoid coccidiosis has also been found in wild circl buntings housed in aviaries as

part of a conservation project (McGill et al., 2010) but the intestinal pathology was not as severe as that observed in the blackbirds.

During the current study, coccidiosis and/or significant helminthosis were also seen in blackbirds and a song thrush out-with rehabilitation centres, but the repeated diagnosis of these conditions in young blackbirds being rehabilitated requires preventive measures by those running such centres. It would be prudent to consider rotating outdoor aviaries that are grassed, or using aviaries with concrete flooring. This would limit access to coccidial oocysts, helminth eggs or larvae that may be present in the soil or in invertebrates that act as intermediate or transport hosts. Routine anthelmintic treatment could be helpful in treating the worms, although Xie and Nevis (2012) reported poor results in using preventive medication against coccidiosis. Nevertheless, McGill et al. (2010) concluded that a combination of antiprotozoal medication, improved hygiene and avoidance of overcrowding contributed to the control of coccidiosis in translocated ciril buntings, an approach worth trying in immature blackbirds undergoing rehabilitation.

Disease associated with schistosome-like eggs was seen in two adult blackbirds, and similar eggs were found in the intestinal mucosa or contents of other blackbirds, dunnocks, a redwing, house sparrow, tawny owl and sparrowhawk. Avian schistosomes are trematodes (flukes) belonging to the family Schistosomatidae, the adults of which inhabit the blood vessels of the viscera or nasal fossae (Huffman and Fried, 2008). The eggs produced by the adults pass from the capillaries to the liver and digestive tract, and are passed out in the faeces. Each egg contains an embryo, the miracidium, which hatches in water and enters an intermediate host, often a snail. More work such as molecular identification is required to confirm that the eggs observed in the current study are schistosome eggs, but if confirmed, this would be the first report of such eggs in UK passerines. Horak et al. (2015) stated that most avian schistosomes that used freshwater snails as intermediate hosts belonged to the genera *Trichobilharzia*, *Dendritobilharzia*, *Bilharziella* and *Gigantobilharzia*. Schistosomes of the genera *Dendritobilharzia*, *Bilharziella*, and most species of *Trichobilharzia*, are found in

waterfowl, but *Gigantobilharzia* spp. (and a few *Trichobilharzia* spp.) can be found in passerines (Horak et al., 2015). *Gigantobilharzia huronensis* is widespread in passerine birds in the USA (Brant et al., 2011), and Rudolfova et al. (2006) noted that *Trichobilharzia* sp. was found in a passerine bird in Europe, a pied wagtail (*Motacilla alba*). If the parasite eggs observed in the passerines in the current study were, indeed, schistosome eggs, they would therefore most likely be *Gigantobilharzia* sp. or *Trichobilharzia* sp.

Adult *Gigantobilharzia* spp. and *Trichobilharzia* spp. are located in the hepatic portal vein, mesenteric vein and intestinal vein, and some species of *Trichobilharzia* are found in the veins of the nasal fossae (Huffman and Fried, 2008). Both genera use freshwater pulmonate snails of the family Physidae as intermediate hosts, and *Trichobilharzia* spp. also use snails of the family Lymnaeidae (Horak et al., 2015). Snails of both these families can be found in the UK, and the bladder snail *Physella acuta*, the intermediate host for *G. huronensis* and some *Trichobilharzia* spp. in the USA (Horak et al., 2015), has in recent years become established in aquaria and outdoor ponds in the UK (National Museums of Northern Ireland, 2010). Birds usually become infected when cercariae leave the intermediate hosts (snails) and penetrate the skin of the definitive hosts (birds), but the cercariae may also be ingested (Horak et al., 2015); the blackbirds, dunnocks and other species with schistosome-like eggs in the current study could have acquired the infection by either route.

Pathology in birds associated with schistosomes often takes the form of granulomatous reactions to the eggs in the liver and intestine (Huffman and Fried, 2008; Horak et al., 2015), similar to the pathology observed in the blackbirds in the current study. Globally, avian schistosomes are important because their cercariae can invade human skin, causing the condition “cercarial dermatitis (CD)” or “swimmer’s itch” (Kolarova et al., 2013). Although usually associated with cercariae of *Trichobilharzia* spp. (Kolarova et al., 2013), in the USA *G. huronensis* is also a cause of cercarial dermatitis (Strohm et al., 1981). There have been relatively few recorded outbreaks of CD in the UK, but one

outbreak in Scotland in 2006 was associated with *Trichobilharzia* sp. (Fraser et al., 2008). Lawton et al. (2014) noted that there had been an increase in cases of CD in North America and Europe, and that the condition was now considered to be an emerging infectious disease (EID). In light of the demonstration of schistosome-like eggs in passerine birds in the current study, and the global increase in CD in humans, further identification of these eggs using molecular techniques should be undertaken, to evaluate the potential zoonotic implications. In future it would be worth routinely collecting, examining and storing fixed and frozen intestine, liver and other tissues from blackbirds and dunnocks, and performing more detailed necropsies in birds with such eggs in an attempt to locate and identify the adult parasites.

8.4.4 Fledgling CNS disorder

This was the commonest condition seen in starlings during this study, and was remarkably consistent in the age of bird, time of year and the findings on brain histopathology. All of the cases were seen in May and June, in young starlings around the age they were due to fledge (approximately three weeks old). This could be consistent with a vertically-transmitted agent, for example the virus of avian infectious encephalomyelitis typically causes neurological signs in vertically-infected domestic fowl chicks in the first week of life, and in weeks 2-5 if horizontally infected (Gough and McNulty, 2008). Alternatively, the age distribution might reflect early exposure to a pathogen, such as *Aspergillus fumigatus*, affecting the lungs and sometimes brain of birds in the first three weeks of life (Brown et al., 2008), or exposure to *Enterococcus hirae* that can cause CNS signs, including torticollis, in chicks in the first two weeks of life (Smyth and McNamee, 2008). The consistently young age of affected starlings could also indicate evidence of immunodeficiency, for example chicken anaemia virus, a vertically-transmitted circovirus, is immunosuppressive and results in mortality in chicks from around two weeks of age (Todd and McNulty, 2008).

The other consistent feature was the non-suppurative encephalitis seen on brain histopathology, suggestive of a viral, mycoplasmal or protozoal aetiology. Financial

constraints limited the extent of virology carried out, but no evidence was found of the involvement of flavivirus, avian influenza virus, paramyxovirus, or avian infectious encephalomyelitis virus. A reovirus was recovered from two out of three birds, and initially was thought to be of doubtful significance because reoviruses can be found in healthy birds and are not usually associated with CNS signs. However Lawson et al. (2015a) demonstrated avian reovirus infection in a magpie in the UK, and noted that neurological disease had been reported in a hooded crow with reovirus infection in Finland (Huhtamo et al., 2007). Reoviruses can also be transmitted vertically, and the possible significance of the reovirus isolated from the fledgling starlings with CNS signs is therefore worthy of greater investigation, for example using in situ hybridisation on sections of brain to see if the reovirus is associated with the non-suppurative encephalitis.

More specific testing for other viruses that might cause CNS signs in birds could also be undertaken, including Borna disease (Berg et al., 2001) and alphaviruses such as Buggy Creek virus and Eastern and Western equine encephalitis virus (O'Brien et al., 2010). Tissues from affected starlings have been retained in the deep freeze, in formol saline and in wax blocks should an opportunity arise in the future to test for such viruses. The possible role of a circovirus could be suggested by the age of the affected birds and the presence of bursal atrophy in some birds, and in 2004 (as part of a different study) intestine from one affected starling was found to be strongly positive by PCR for pigeon circovirus and intestine from another was weakly positive. Two years later a novel circovirus of starlings in Spain was described by Johne et al. (2006), raising the possibility of the presence of a starling circovirus in Scotland that cross-reacted on PCR with pigeon circovirus. This is another area worthy of further investigation in the future.

Mycoplasma gallisepticum has been shown to cause a non-suppurative encephalitis in turkeys (Chin et al., 1991) but was not detected in affected starlings in the current study. *M. sturni* was, however, isolated from all eight affected birds tested, but not from two adults that had died from pox and trauma respectively. To further investigate the

potential role of *M. sturni*, techniques such as immunolabelling of fixed tissues could be attempted, to see if the mycoplasmas were associated with the brain lesions.

Another possible explanation for the non-suppurative encephalitis observed on histopathology would be a protozoal infection. No protozoal bodies were detected in the brains reported on by Pennycott* et al. (2002b), but small numbers of structures believed to be protozoal schizonts were subsequently found in the brain of an affected starling and an affected house sparrow in the current study. Possible protozoa that could affect the brain of animals and birds include those of the family Sarcocystidae such as *Neospora caninum*, *Toxoplasma* spp. and *Sarcocystis* spp. *Neospora* and *Toxoplasma* are two closely-related genera of protozoa whose definitive hosts are canids and felids respectively and which can cause abortion in cattle (*Neospora*) and sheep (*Toxoplasma*). Evidence of exposure to *Toxoplasma* spp. has been found in apparently healthy starlings in England (Peach et al., 1989) and in sparrows in Poland and the Czech Republic (Literak et al., 1997a). More recently, the DNA of *Neospora* and/or *Toxoplasma* has been identified in the brain of house sparrows caught in Brazil (Gondim et al., 2010), and in various raptors and corvids in Colorado, USA and Spain (Dubey et al., 2010; Darwich et al., 2012).

Sarcocystis spp. have been implicated in neurological disease in wild birds, including capercaillie in Sweden (Gustafsson et al., 1997), a northern gannet in Florida, USA (Spalding et al., 2002), and a feral pigeon from Minnesota, USA (Wunschmann et al., 2011). The protozoal schizonts illustrated in the papers relating to capercaillie and gannet looked very similar to those observed in the starling and house sparrow in the current study, but the histopathological lesions varied between different species of bird. In the cases described in capercaillie, there was a non-suppurative meningo-encephalitis, especially of the cerebellum, compared to the non-suppurative encephalitis mostly affecting cerebrum and midbrain of starlings. Protozoal schizonts were observed in over two-thirds of the affected capercaillie, whereas in starlings they were rarely observed. Protozoal schizonts and merozoites were numerous in the brain of the gannet with

Sarcocystis sp., and tissue cysts were widely distributed in the cardiac and skeletal muscle, but no muscle cysts were observed in the starlings. In the feral pigeon with sarcocystosis, large numbers of tissue cysts were detected in the skeletal muscle but no protozoal stages were found in the brain, and the inflammatory response was granulomatous rather than non-suppurative as observed in starlings. It appears that different species of birds respond to *Sarcocystis* spp. in different ways. Insufficient testing for *Neospora*, *Toxoplasma* or *Sarcocystis* has been carried out on tissues from starlings with fledgling CNS disorder due to limited resources and lack of availability of a suitable antigen for detecting *Sarcocystis* spp., but if possible this should be addressed in the future.

In summary, the cause of the fledgling CNS disorder of starlings (and house sparrows) remains to be determined, but fixed and frozen tissues from affected birds have been retained in case the opportunity arises in the future to explore the possible roles of avian reovirus, starling circovirus, *M. sturni*, protozoa, or other potential aetiological agents.

8.4.5 Mycotic pneumonia / airsacculitis

Mycotic pneumonia/airsacculitis caused by *Aspergillus fumigatus* was found in several immature blackbirds. Jansson (2012) suggested that most cases of aspergillosis in free-living birds involved waterfowl, gulls and birds of prey, but wildlife rehabilitators and their veterinary advisers should be aware that immature blackbirds are also susceptible to aspergillosis. Rosen (1964) described an incident in two walnut orchards in the USA in which “hundreds” of passerines were found dead. Of twenty birds submitted for necropsy, 15 were American robins (family Turdidae) and all had aspergillosis, underlining the susceptibility of birds of the family Turdidae to this condition. Friend and Trainer (1969) concluded that young herring gulls acquired low-level chronic aspergillosis from the environment early in life, with disease subsequently escalating in some birds due to additional stress factors, and the same may be true for blackbirds and related birds. Wildlife rehabilitators need to be aware of the susceptibility of young

blackbirds to aspergillosis, and must take the appropriate steps to reduce stress and exposure to pathogenic fungi.

Chapter 9

Diseases of UK corvids

9.1 Introduction

The family Corvidae (crows) includes commonly-seen birds such as the rook, carrion crow (*Corvus corone*), hooded crow (*C. cornix*, once considered to be a subspecies of the carrion crow but now classified as a separate species), jackdaw and magpie. The family also includes the much larger raven (*C. corax*), the brightly-coloured jay (*Garrulus glandarius*), and the endangered chough. The Latin names of the UK birds discussed in this chapter are listed in Appendix I.

There are over one million breeding pairs of rooks and of carrion crows in the UK, and approximately 1.4 million pairs of jackdaws (Musgrove et al., 2013). Smaller numbers of magpies (600,000 breeding pairs), jays (170,000 pairs) and hooded crows (160,000 pairs) are present in the UK, and a little over 7,000 breeding pairs of ravens (Musgrove et al., 2013). In contrast, the UK breeding population of choughs is much smaller, numbering fewer than 400 breeding pairs, and the chough is amber-listed as being of conservation concern in the UK (Hayhow et al., 2015). In Scotland there are fewer than 60 breeding pairs of choughs, mostly on the island of Islay (Hayhow et al., 2015). Corvids in the UK are essentially sedentary, but some birds from the continent visit in winter, especially jackdaws (Henderson 2002), and large irruptions of jays into the UK have been recorded when the acorn crops in northern Scandinavia and Russia have failed (Wilson 2002).

9.2 Review of diseases found in UK corvids

(References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.)

9.2.1 Trauma

Many of the corvids are regarded as pest species by farmers and gamekeepers, and the Wildlife and Countryside Act of 1981 permits the killing by authorised persons, under some circumstances, of rooks, carrion crows, jackdaws, magpies and jays. It is not surprising, therefore, that “deliberately taken by man” was the commonest reason recorded by the British Trust for Ornithology when these birds had been rung and the carcasses subsequently recovered. 79% of carrion/hooded crows (O’Donoghue 2002), 77% of rooks (Brenchley 2002), 74% of jays (Wilson 2002), 65% of magpies (Cannon 2002) and 63% of jackdaws (Henderson 2002) fell into this category. Even the raven, which is legally protected, was frequently deliberately killed (63% of ring recoveries, Cross 2002).

9.2.2 Poisoning

Together with raptors, corvids are the birds most likely to be involved in deliberate poisoning incidents. Jennings (1961) found that trauma and poisoning (unspecified) were responsible for the deaths of most of the corvids he examined between 1952 and 1959, and Keymer (1958) reported suspected or confirmed dieldrin poisoning in rooks and jackdaws. Strychnine poisoning of a magpie and raven were listed by Baker (1977), and nearly twenty years later Johnson (1996) highlighted 91 poisoning incidents in corvids between 1990 and 1994, commenting that corvids are probably the commonest targets of deliberate abuse. Examination of the annual reports “Pesticide Poisoning of Animals – Investigations of Suspected Incidents in the UK” for 1998 to 2004, produced by MAFF and then Defra as part of the Wildlife Incident Investigation Scheme, showed that deliberate poisoning of corvids was still a problem (WIIS 1998-2004). During these seven years there were nearly 50 confirmed incidents of poisoning in corvids. The pesticides most commonly abused were carbofuran, alphachloralose, mevinphos and

bendiocarb. Most incidents were confirmed in small numbers of birds but some involved multiple deaths, for example five magpies (strychnine), 22 ravens (fenthion), 18 jackdaws (alphachlorase) and 30 rooks (mevinphos). Accidental poisoning can also occur; 18 magpies died in two incidents after a pour-on preparation of famphur had been applied to cattle to treat warble flies (Felton et al., 1981). Magpies were thought to be particularly at risk because of their habit of feeding on the backs of cattle.

9.2.3 Malnourished fledglings

Stocker (2000) noted that members of the public frequently presented corvid fledglings to wildlife rehabilitation centres because they mistakenly believed that the birds had been abandoned, unaware that young corvids leave the nest before they can fly and are still fed by their parents while on the ground. The great majority of these birds are healthy, but some show signs of malnutrition such as feathers that have pale patches and are easily broken, poor size, and deformed legs (Stocker 2000). This is partly because young corvids hatch in an asynchronous fashion and the last to hatch often receive less food than those that hatched earlier, become smaller and weaker than their hatch mates and eventually die, especially in years of limited food supply (Coombs 1978). Mortality from malnutrition in young rooks can continue from June to August due to a bottleneck in food availability (Brenchley 2002).

9.2.4 Internal parasites – helminths and coccidia

Probably the best known infectious disease of corvids is helminthosis, especially caused by burdens of the gapeworm *Syngamus trachea* in the larynx, trachea and syrinx. Elton and Buckland (1928) found that 94% of recently fledged rooks in May had burdens of *S. trachea* and commented that most were infected to a fairly heavy extent, with over 40 pairs of worms found in one bird. These authors concluded that gapeworm burdens were very likely to cause mortality in young rooks prior to fledging. In contrast, only small numbers were found in adult rooks, but nevertheless infected birds posed a threat to the health of free-range poultry. Campbell (1935) identified *S. trachea* in rooks (82.0%), carrion crows (36.3%), magpies (33.3%) and jackdaws (13.8%), and commented that the

trachea of many of the young rooks was considerably inflamed, even in birds with relatively small numbers of worms. Of 372 fledgling rooks that had been shot, Clapham (1957) found *S. trachea* in 369 birds (99.2%), and experimental evidence that *S. trachea* from corvids and starlings could spread to poultry and gamebirds through infected earthworms was presented by Clapham (1940). All the above authors observed that gapeworm burdens were highest in young birds, reducing as the birds grew older. Infection with *S. trachea* has also been shown to cause respiratory distress and mortality in young and adult choughs (Bignal et al., 1987; Meyer and Simpson, 1988). Adult *S. trachea* are typically found as pairs in permanent copulation but the sexes of a similar parasite, *Cyathostoma lari*, are not joined together (Fernando and Barta, 2008). This worm can be found in the orbital sinuses of some species of birds including rooks, carrion crows and jackdaws (Pemberton 1959) but have not been associated with clinical disease.

Another group of nematodes described in corvids are hairworms of the subfamily Capillariinae; Clapham (1957) demonstrated *Capillaria contorta* in the upper digestive tract of all rooks examined and in 89% of magpies, and Keymer et al. (1962) recorded *Capillaria retusa* (now considered a synonym for *C. anatis*) in the intestine of two rooks and a jackdaw. None of the above authors described clinical disease in corvids associated with hairworms. The classification of hairworms is difficult and unsatisfactory (Anderson 1992) and nomenclature has changed. Yabsley (2008) listed *Eucoleus dispar* and *Eucoleus contortus* as occurring in the upper digestive tract of corvids, and *Capillaria anatis* and *Baruscapillaria resecta* in the intestine. Barus and Sergejeva (1989) concluded that hairworms found in the upper digestive tract of corvids were *E. dispar*, as distinct from *E. annulatus* from the upper digestive tract of gallinaceous birds and *E. contortus* from the upper digestive tract of aquatic birds. Anderson (1992) described the hairworms found in the upper digestive tract of corvids as *E. contortus* and *E. frugilegi*. To avoid confusion, and in the absence of detailed examination of the parasites, it is proposed in the current study to refer to hairworms of

the upper digestive tract of corvids as *Eucoleus* sp., and those of the intestine as *Capillaria/Baruscapillaria* sp.

Tapeworms of the genus *Hymenolepis* were found by Clapham (1957) in a high proportion of rooks, magpies and jackdaws, and in a review of parasites found in wild birds in the UK, Keymer et al. (1962) included the tapeworm *Dilepis undula* in the rook and jackdaw. Clapham (1957) also found the large roundworm *Porrocaecum ensicaudatum* in immature (but not adult) rooks, and the eggs of this parasite were described in detail by Wharton (1979). Thorny-headed worms identified as *Polymorphus boschadis* were demonstrated by Clapham (1957) in 55% of immature rooks and 94% of adult rooks. [*Polymorphus boschadis*, now referred to as *Polymorphus minutus*, is a parasite of waterfowl and it is possible that the parasites found in corvids were actually *Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus*, a cosmopolitan species found in passerines (Richardson and Nickol, 2008)]. None of the above parasites were linked to clinical disease. Jennings and Soulsby (1957) noted the presence of *Syngamus trachea*, *Porrocaecum ensicaudatum* and *Prosthorhynchus rostratus* (now considered synonymous with *Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus*) as incidental findings in a rook.

Intestinal coccidia described as *Isospora* sp. were found in five of 23 rooks examined by Keymer et al. (1962) and judged to be incidental findings. Schrenzel et al. (2005) proposed that all such coccidia should be described as “isosporoid coccidia” rather than *Isospora* sp. or the morphologically similar *Atoxoplasma* sp. – see section 3.2.9 of this thesis for further details.

9.2.5 Pasteurellosis and corvid respiratory syndrome

Deaths from *Pasteurella multocida* infection have been described in garden birds that have been injured by cats (see 3.2.2 and 8.2.2 of this thesis). In addition, *P. multocida* has been demonstrated in corvids dying with evidence of respiratory disease (Strugnell et al., 2011). These authors examined carrion crows and rooks found dead or trapped

alive on an outdoor pig unit where large numbers of corvids had been found dead or observed showing signs such as dyspnoea, snicking, coughing and inability to fly. Postmortem findings in birds found dead or trapped alive included combinations of fibrinous pericarditis, perihepatitis and airsacculitis of varying severity, sinusitis, and focal or necrotic pneumonia. *P. multocida* was recovered from the tissues of all five birds submitted alive and with lesions of respiratory disease, but not from any of twelve birds found dead and with pathology of the respiratory tract. However, histopathological examination of the lungs and/or airsacs of most of the birds found dead had pneumonia or airsacculitis associated with Gram-negative coccobacilli that were positively labelled by immunohistochemistry for *P. multocida*. Four isolates of *P. multocida* were subsequently shown to be capsular serogroup F, with a number of different somatic serotypes. Various mycoplasmas including *M. sturni*, *M. gallinarum*, *M. gallopavonis* and *M. colomborale* were isolated from some birds but were not considered significant, nor were cross-sections of nematodes in the lung of three birds and of a trematode in the lung of one bird. Although Strugnell et al. (2011) concluded that *P. multocida* was the cause of the respiratory disease observed in the corvids, they also expressed surprise that the bacterium was not isolated from birds found dead, and that the isolates from live birds were serogroup F rather than serogroup A which is more frequently reported from birds. Further cases of airsacculitis and pneumonia in corvids associated with *P. multocida* were seen in 2013 (Anon 2013a).

The above papers were not the first descriptions of outbreaks of respiratory disease in corvids; McDiarmid (1956) noted that “a considerable number of rooks, crows, jackdaws and starlings have been found with extensive lesions of the airsacs and lungs”. He considered the cause to be *Pseudomonas aeruginosa* (then referred to as *Bacillus pyocyaneus*) which had been isolated from most of the carcasses, but wondered if other agents were also involved. Keymer (1958) also reported extensive airsacculitis in a rook, but no cause was given. It is possible that the characteristic combinations of postmortem features described by different investigators have more than one cause, and in this thesis the term “corvid respiratory syndrome” will be used.

9.2.6 Infection with *Mycobacterium avium* and *Yersinia pseudotuberculosis*

There are few reports of avian tuberculosis in corvids caused by *Mycobacterium avium* subspecies *avium*, and although McDiarmid (1956) indicated that he had found the disease in 1% of rooks and jackdaws, no further information was provided. Keymer (1961) noted extensive lesions of avian tuberculosis, confirmed by culture, in a thin jackdaw found on a poultry farm. The closely related *M. a. paratuberculosis*, the cause of Johne's disease in cattle and sheep, was cultured from the tissues of multiple corvids collected as part of vermin control on farms in Scotland with Johne's disease; Beard et al. (2001) recovered the organism from 36/60 carrion crows, 3/54 rooks and 1/38 jackdaws. In only one bird, a carrion crow, was there any histological evidence to suggest that *M. a. paratuberculosis* may have become established in the digestive tract, but these authors concluded that corvids (and rats, mice and hares) could passively disseminate the organism causing Johne's disease to cattle and sheep.

Focal necrosis of the liver and spleen of many species of birds, including corvids, can result from infection with *Yersinia pseudotuberculosis* (Mair 1973). Bullock et al. (1983) commented that choughs are susceptible to pseudotuberculosis, and Macdonald (1962b), when discussing pseudotuberculosis in wild birds, noted that disease associated with lameness may have contributed to the decline of choughs in Cornwall.

9.2.7 Avian pox, cnemidoptotic mange and other skin conditions

Avian pox affecting the legs of a carrion crow was described and illustrated by Poulding (1960); numerous wart-like growths up to 1.5 cm in diameter were present on the legs and feet, and the diagnosis was confirmed by histopathology. Pennycott* (2003) reported finding cnemidoptotic mange (scaly leg) in a rook, carrion crow and jackdaw, in which a layer of white or grey debris was found on the hock, shank or foot. Microscopy revealed *Cnemidocoptes* sp. (synonymous with *Knemidocoptes* sp.) mites, most likely *C. jamaicensis*. Keymer and Blackmore (1964) also noted a case of scaly leg in a rook but ascribed the cause to *Cnemidocoptes mutans*, the cause of scaly leg in

poultry. Coombs (1960) listed the lice, feather mites, hippoboscids flies and other external parasites found in 100 rooks and 40 jackdaws, but made no mention of any associated pathology. Unidentified mites that spread from corvids in rehabilitation centres to people handling the birds were highlighted by Stocker (2000).

9.2.8 Infection with *Salmonella* spp., *Campylobacter* spp. and *Chlamydia* (*Chlamydophila*) *psittaci*

Salmonella spp., *Campylobacter* spp. and *Chlamydia* (*Chlamydophila*) *psittaci* all have the potential to spread from birds and cause clinical disease in humans. Unlike finches and sparrows (Chapter 4), salmonellosis is not a common cause of death in corvids, and when recovered from corvids is usually as an incidental finding, reflecting contamination of the environment from other sources. For example, Harbourne (1955) isolated *S. Gallinarum* (the causal agent of fowl typhoid in poultry) from 7 out of 42 rooks from parts of England in which fowl typhoid was common, and Jones et al. (1983) isolated *S. Saint-Paul* from the feet, oropharynx or cloaca of four rooks and a carrion crow caught during an outbreak of salmonellosis in a cattle herd. *S. Typhimurium* was isolated from two rooks by Pennycott* et al. (2006), but only through enrichment media and not from the other 53 corvids screened.

Corvids have been implicated in the spread of *Campylobacter* spp. to sheep and to humans. Watson et al. (1967) explored the possible role of carrion crows in introducing *Campylobacter fetus* (then described as *Vibrio fetus*) into sheep flocks where it could result in abortion. They found spirochaetes resembling *C. fetus* in a high proportion of faeces or intestinal mucosal smears made from live-trapped crows, and isolated *C. fetus* from three crows. When the isolate from crows was inoculated into four pregnant sheep, abortion resulted in all four, and the authors concluded that on some occasions crows could introduce this pathogen to sheep flocks. In the early 1990s, magpies and jackdaws were believed to be the source of *C. jejuni* food poisoning in some humans, by pecking or tearing off the foil tops of milk delivered to households and left on the doorstep (Southern et al., 1990; Hudson et al., 1991). Supporting evidence was presented by

Simpson et al. (1991) who recovered *Campylobacter* spp. from the oropharynx or cloaca of 29 out of 37 magpies that had been shot, trapped or were road kills, and Hudson et al. (1991) isolated *Campylobacter* spp. from the oropharynx or cloaca of 33 out of 35 corvids (jackdaws, magpies, rooks and carrion crows) and from the milk of 12 out of 123 bottles with pecked tops. In contrast, Hughes et al. (2009) recovered *Campylobacter* spp. from faecal samples from only 4 out of 80 corvids, possibly reflecting different screening methodologies. Although it appears that a high proportion of corvids carry *Campylobacter* spp., with the exception of the cases of human food poisoning arising from corvids pecking or removing foil tops from milk bottles, modern molecular methods such as multilocus sequence typing have indicated that most cases of *Campylobacter* food poisoning in humans are acquired from poultry meat or ruminant sources (Sheppard et al., 2009).

Chlamydia psittaci has most often been demonstrated in pigeons and doves (Gough and Bevan, 1983; Sharples and Baines, 2009) and robins (Simpson and Bevan, 1989; Colville et al., 2012; Beckmann et al., 2014a) but the organism has also been recovered from rooks with respiratory disease (Pennycott* et al., 2009). No large surveys have been carried out to establish the prevalence of infection in corvids in the UK, but a study in Italy demonstrated chlamydiae from 22 out of 76 cloacal swabs collected from magpies and hooded crows (Di Francesco et al., 2015). Sequencing subsequently showed that only one swab yielded *C. psittaci*, the sequences from the remaining 21 samples were identical to those of *C. suis*. These authors suggested that the corvids had acquired *C. suis* from wild boar in the area from which the corvids were sampled.

9.2.9 Infection with avian influenza virus, avian paramyxovirus, West Nile virus and avian reovirus

Low pathogenic strains of avian influenza virus (LPAIV) have been recovered from many species of birds globally, especially waterfowl, gulls and waders. In recent years a highly pathogenic strain of avian influenza virus (HPAIV) designated H5N1 has circulated in Europe and elsewhere, causing mortality in poultry, humans and wild birds.

In addition to the bird groups already mentioned, HPAIV H5N1 was detected in corvids and raptors, possibly acquired by feeding on the carcasses of other birds that had died from HPAIV (Reperant et al., 2012). However, no records were found of the recovery of this virus from corvids in the UK.

Another virus that can cause high mortality in poultry is avian paramyxovirus-1 (APMV-1, Newcastle disease virus). Kaleta (2012) considered that all species of wild bird in Europe were susceptible to APMV-1, and Keymer (1961) described the recovery of this virus from a jackdaw in the UK. The bird had been found on a poultry farm on which Newcastle disease had been confirmed, and was thin and unable to fly. Postmortem examination showed the spleen to be enlarged and haemorrhagic, marked thickening of the airsacs was present and the lungs were oedematous. APMV-1 was subsequently isolated from the viscera, feathers and feet of the bird. However when the virus was then fed back to wild jackdaws, the birds seroconverted but remained clinically healthy. The author concluded that the clinical signs and pathology found in the sick jackdaw may have been caused by something other than APMV-1, and that corvids in general were not clinically susceptible to APMV-1 but could spread disease to poultry flocks.

West Nile virus (WNV) is a flavivirus spread by mosquitoes, and can affect humans, horses and birds including wild birds. Most infected humans show no clinical signs, but some develop mild fever, headaches and a skin rash. Neurological signs associated with meningitis or encephalitis are seen in a very small proportion of infected humans. Infected horses can also develop fever and neurological signs. WNV unexpectedly appeared in North America in 1999, and in addition to causing disease in humans and horses resulted in the deaths of thousands of wild birds, especially birds of the family Corvidae (Rappole et al., 2000). Affected birds frequently showed neurological signs prior to death, and histopathology often demonstrated meningo-encephalitis and myocarditis (Steele et al., 2000). Disease caused by WNV has also been seen in humans, geese and horses in Europe (Phipps et al., 2008), and in 2012 there were over 220

confirmed human cases in Bulgaria, Greece, Hungary, Italy and Romania (ECDC 2014). Unlike the picture seen in North America, WNV has not caused mass mortality in wild birds in Europe (Phipps et al., 2008). There have been no confirmed UK-acquired cases of WNV infection in humans or horses, nor has disease been confirmed in wild birds in the UK (Phipps et al. 2008). However one study (Buckley et al., 2003) reported the demonstration of antibodies to WNV in wild birds in England and Wales. Nearly 30% of 70 carrion crows and magpies (and 20% of 41 blackbirds) were seropositive using a plaque reduction neutralisation test with a cut-off point of 90% plaque neutralisation, and a nested RT-PCR and sequencing detected WNV RNA in the brain or serum of six magpies and a blackbird. Attempts to culture the virus on Vero cell monolayers were, however, unsuccessful. The authors concluded that different strains of WNV were circulating in wild birds in the UK, and that the presence of antibodies could perhaps protect against the neurological signs and deaths observed in birds in North America.

Severe necrosis of the liver and spleen was present in an adult magpie found moribund in England (Lawson et al., 2015a). A reovirus was subsequently isolated from pooled liver/kidney and from intestinal contents, and in situ hybridisation using a biotin-labelled probe for avian reovirus demonstrated positive labelling associated with the hepatic and splenic necrosis. Although this was believed to be the first report of reovirus-associated mortality in wild birds in the UK (Lawson et al., 2015a), fatal necrotising enteritis associated with an avian reovirus has been reported in corvids in Canada and the USA (Campbell et al., 2004; Meteyer et al., 2009). Avian reovirus of unknown significance has also been isolated from fledgling starlings showing CNS signs in the UK, and corvids are known to prey on starlings (see 8.3.10 and 8.4.4 of this thesis). In future it would be worth comparing isolates of reoviruses from corvids with those from starlings.

9.2.10 Miscellaneous conditions and pathogens

Beak deformities

Beak deformities such as crossed mandibles and decurvature of the upper mandible were described in corvids by Pomeroy (1962), and Coombs (1978) noted that there had been several reports of beak deformities in corvids.

Screening for Mycoplasma spp.

Oropharyngeal and conjunctival swabs from 23 corvids from Scotland were screened for mycoplasmas by culture, and by PCR for *M. gallisepticum* and *M. synoviae* (Pennycott* et al., 2005b). *M. sturni* was isolated from ten birds that had died from a range of conditions including malnutrition, parasitism, encephalitis and trauma. *M. sturni* has also been isolated from corvids with respiratory disease (Strugnell et al., 2011) but is of doubtful significance. *M. gallisepticum* was not isolated from any corvid but was detected by PCR in four rooks with respiratory disease (Pennycott* et al., 2005b); a study reported by Bradbury et al. (2000) had previously found a high proportion of healthy rooks and carrion crows, and to a lesser extent jackdaws, to be PCR-positive but culture-negative for *M. gallisepticum*. On the basis of these results it was unclear if corvids might provide a reservoir of *M. gallisepticum* that could jeopardise the health of poultry and gamebirds. None of the samples were positive by PCR for *M. synoviae* (Bradbury et al., 2000; Pennycott* et al., 2005b).

Adverse environmental conditions

Coombs (1978) reported that, unlike some groups of birds, corvids did not suffer heavy mortality during extended spells of very cold weather because of their adaptable omnivorous diet.

Haemoparasites

Haemoparasites spread by biting insects have been reported in corvids. Baker (1982) noted that in one area of England, 5% of wild birds (rooks, jackdaws and blackbirds) were infected by *Plasmodium* spp., spread by biting mosquitoes, and 15% of adult rooks

carried *Leucocytozoon* spp. (transmitted by black flies of the family Simuliidae). However there was no evidence that they caused disease in the birds. Similarly, *Haemoproteus* spp., spread by ceratopogonid flies (midges) and hippoboscids (louse flies), have been found in healthy corvids (Bishop and Bennett, 1990), and *Trypanosoma corvi*, spread by louse flies, was said to infect about 30% of healthy rooks and jackdaws (Baker 1982).

9.3 Diseases of corvids examined at Ayr DSC 1994 to 2013 – results

9.3.1 Bird species, numbers and locations

Two hundred and ninety-two corvids were examined between January 1994 and December 2013. All of the choughs were found on the islands of Islay or Oronsay. Of the remaining corvids for which a location was given, 95% were found in the south of Scotland and 5% in the north. A summary of the species examined and the conditions diagnosed can be found in Tables 9.1 – 9.3. The materials and methods are described in Chapter 2 and the diagnostic criteria used are listed in Appendix II.

Table 9.1: Corvids examined.

Species	Number examined
Rook	105
Carrion crow	69
Hooded crow	5
Chough	42
Jackdaw	36
Magpie	28
Raven	7
Total	292

Table 9.2: Conditions* diagnosed in corvids (excluding choughs) (n=250).

Condition	Number of birds	% (95% confidence intervals)
Trauma	81	32.4 (26.7-38.6)
Malnourished fledglings	54	21.6 (16.8-27.3)
Significant helminthosis	54	21.6 (16.8-27.3)
Significant numbers of external parasites (excluding cnemidocoptic mites)	23	9.2 (6.0-13.7)
Respiratory tract condition not otherwise specified	23	9.2 (6.0-13.7)
Corvid respiratory syndrome	20	8.0 (5.1-12.3)
Arthritis and/or cellulitis	14	5.6 (3.2-9.4)
Digestive tract condition not otherwise specified	10	4.0 (2.0-7.5)
Chlamydiosis/chlamydiasis	9	3.6 (1.8-6.9)
Miscellaneous conditions and pathogens:		All <3%
Adverse environmental conditions	5	
Central nervous system condition not otherwise specified	3	
<i>Pasteurella multocida</i> infection	4	
Mycotic pneumonia / airsacculitis	4	
Pox (suspected or confirmed)	4	
Mycobacteriosis	2	
Cnemidocoptic mange (scaly leg)	5	
Necrotic enteritis	1	
Feather/skin abnormality not otherwise specified	3	
Beak deformities	3	
<i>Staphylococcus aureus</i> infection	1	
<i>Candida albicans</i> infection	7	
Reproductive tract disorder	1	
Neoplasia	2	
Poisoning	1	
No diagnosis	23	9.2 (6.0-13.7)
Incidental findings:		Not applicable
<i>S. Typhimurium</i> through selenite only	2	
Small to large numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Helminth burdens not considered significant	Not recorded	
Cryptosporidia in the bursa of Fabricius of a magpie	1	
Isolation of <i>M. sturni</i> from oropharyngeal swabs	11	
Tissues screened for avian influenza virus – no positive results	71	
Tissues screened for West Nile virus – no positive results	81	

*Not all birds were examined for all conditions, and some birds had multiple conditions

Table 9.3: Conditions* diagnosed in choughs (n=42).

Condition	Number of birds	% (95% confidence intervals)
Developmental abnormalities of the eyes	14	33.3 (20.0-49.6)
Significant helminthosis	5	11.9 (4.5-26.4)
Digestive tract condition not otherwise specified (necrotic oesophagitis)	5	11.9 (4.5-26.4)
Trauma	3	7.1 (1.9-20.5)
<i>Yersinia pseudotuberculosis</i> infection	3	7.1 (1.9-20.5)
Miscellaneous conditions and pathogens:		All <5%
Adverse environmental conditions	2	
Arthritis	1	
Reproductive tract disorder	1	
Significant numbers of external parasites	1	
No diagnosis	9	21.4 (10.8-37.2)
Incidental findings:		Not applicable
Small to large numbers of coccidial oocysts presumed to be isosporoid coccidia	Not recorded	
Helminth burdens not considered significant	Not recorded	
Isolation of <i>M. gallisepticum</i> from oropharyngeal swabs	2	
Tissues screened for avian influenza virus – no positive results	22	
Tissues screened for West Nile virus – no positive results	24	

*Not all birds were examined for all conditions, and some birds had multiple conditions

9.3.2 Trauma

A diagnosis of trauma was made in 30 rooks, 24 carrion crows, 13 magpies, 10 jackdaws, three hooded crows, three choughs and one raven. Two carrion crows that died from trauma had heavy burdens of gapeworms, another was a malnourished fledgling, one had a large cystic mass on the liver and one had pre-existing damage to one shoulder. Predisposing factors found in rooks included heavy burdens of gapeworms (three birds), malnourished fledgling (one bird) and significant lesions of scaly leg (one bird); and two young jackdaws with head trauma also had mycotic pneumonia/airsacculitis. No predisposing factors were found in the hooded crows, raven or choughs that died from trauma, but one magpie had a pre-existing fractured wing.

Wing injuries and internal haemorrhage were the commonest findings at necropsy, and road traffic accidents were probably the major cause. Head/neck injuries seemed to be especially common in magpies and jackdaws (Image 297). Three corvids were found below pylons or power lines, nine had been shot (Images 294-296), two had been attacked by other crows, and another two had puncture wounds in the breast suggestive of a raptor attack. Cat-inflicted injuries were suspected in three magpies, and five rook nestlings died from trauma after their nests were blown out of the trees during high winds (Image 549). Unlike the situation with garden birds, *P. multocida* was not isolated from any corvids that died from trauma.

9.3.3 Malnourished fledglings

Thirty rooks, 11 carrion crows, eight jackdaws, four magpies and one raven were categorised as “malnourished fledglings”, all in the months May to June except for two jackdaws submitted in August (Images 298-299). Affected birds were thin, often poorly feathered and sometimes with soft rubbery bones. Significant gapeworm burdens were found in many of the malnourished rooks but not in other species, and one carrion crow had many intestinal flukes and necrotic tracts in the liver from which *P. multocida* was isolated. Two malnourished jackdaws were submitted in August of two different years and may have represented replacements for failed earlier broods. Both had soiled vents and fluid intestinal contents, and a PCR for *C. psittaci* was carried out on intestinal contents of one of the birds with positive results.

9.3.4 Mycotic pneumonia / airsacculitis and mycobacteriosis

Mycotic pneumonia/airsacculitis was found in four immature jackdaws, and mycobacteriosis (presumed to be avian tuberculosis) was diagnosed in an adult jackdaw and also concurrently in one of the immature jackdaws with mycotic pneumonia/airsacculitis. No other species of corvid was found to have either of these conditions. One jackdaw was found alive, coughing and showing leg paralysis, and was euthanased. Multiple nodules were present in the lungs and the adjacent airsacs were solid and white/fawn in colour (Image 300). A pale nodule extended into the lumbar

spinal canal (**Images 301-302**). Although no fungi were isolated, histopathology demonstrated septate branching fungal hyphae consistent with *Aspergillus* sp. in heterophilic granulomata in the lungs and airsacs. Two jackdaws were found in the vicinity of their nest sites and had fractured skulls; one had nodules in the lungs and both had plaques on the airsacs. No fungi were isolated but histopathology showed granulomata associated with branching septate fungal hyphae and conidiospores suggestive of *Aspergillus* sp. The fourth immature jackdaw had multiple nodules in the lungs (**Image 303**) from which *Aspergillus fumigatus* was isolated. In addition, the spleen of this bird was greatly enlarged, pale nodules were present in the liver and spleen and on the serosa of the duodenum and gizzard, and large numbers of acid-alcohol-fast bacilli were seen in Ziehl-Neelsen stained smears, indicative of a mycobacterial infection (**Images 304-305**). One thin adult jackdaw had irregular pale areas on the heart, excess pericardial fluid, an enlarged pale liver, and a slightly enlarged nodular spleen with multiple pale foci (**Images 306-307**). Histopathology confirmed a diagnosis of mycobacteriosis, most likely avian tuberculosis.

9.3.5 Helminths and coccidia

The commonest helminths found in corvids during the 20 years of the study were gapeworms (*S. trachea*), hairworms of the upper and lower digestive tract, and tapeworms. The large roundworm *Porrocaecum* sp. and the syngamid worm *Cyathostoma* sp. were found in small numbers of birds, as were thorny-headed worms and unidentified intestinal flukes. Spirurid gizzard worms were found in choughs.

Between 1995 and 1996, the oesophagus, intestine and trachea of 100 corvids were opened and examined grossly for helminths, mucosal smears from the upper and lower digestive tracts were examined microscopically, and the magnitude of the burdens of *S. trachea* recorded. Less detailed parasitological examination was carried out after 1996 due to time constraints. Forty rooks, 26 carrion crows, five hooded crows, 16 magpies, 12 jackdaws and one raven were examined between 1995 and 1996. Table 9.4 summarises the main parasites (gapeworms, hairworms and tapeworms) found in

different categories of corvid, and Table 9.5 shows the numbers of *S. trachea* found. Birds were classified as “immature” if nestling, fledgling or in their first summer up to August, when they would have finished their first moult and become independent of their parents (all immature birds were actually received in April to June). “Mature” included first-year birds after August and older birds.

Table 9.4: Numbers of birds* with gapeworms, hairworms and tapeworms (1995-1996).

Type of bird	Number of birds examined	Number with gapeworms	Number# with hairworms	Number with tapeworms
Rook - immature, April to June	20	19	4	17
Rook - mature, April to June	13	7	6	4
Rook - mature, remaining nine months	7	1	4	1
Carrion crow - immature, April to June	7	5	1	3
Carrion crow - mature, April to June	5	3	0	3
Carrion crow - mature, remaining nine months	14	3	10	4
Hooded crow - immature, April to June	0	0	0	0
Hooded crow - mature, April to June	1	1	0	0
Hooded crow - mature, remaining nine months	4	2	2	1
Magpie - immature, April to June	2	0	0	0
Magpie - mature, April to June	5	1	0	0
Magpie- mature, remaining nine months	9	3	2	1
Jackdaw- immature, April to June	4	1	0	1
Jackdaw - mature, April to June	4	3	1	2
Jackdaw- mature, remaining nine months	4	1	2	0

* excluding one raven, in which no helminths were detected.

mostly of the upper digestive tract

Table 9.5: Numbers of *S. trachea* in different categories of bird (1995-1996).

Category	Number of pairs of <i>S. trachea</i>				
	None	1-10	11-20	21-50	> 50
Rook - immature, April to June	1	4	4	5	6
Rook - mature, April to June	6	4	1	2	0
Rook - mature, remaining nine months	6	1	0	0	0
Carrion crow - immature, April to June	2	3	1	1	0
Carrion crow - mature, April to June	2	1	1	1	0
Carrion crow - mature, remaining nine months	11	2	0	1	0
Mature hooded crow (all twelve months)	2	3	0	0	0
Immature magpie (all twelve months)	2	0	0	0	0
Mature magpie (all twelve months)	10	4	0	0	0
Immature jackdaw (all twelve months)	3	1	0	0	0
Mature jackdaw (all twelve months)	4	4	0	0	0

The worm most frequently detected in 1995-1996 was *S. trachea*, found in the larynx, trachea or bronchi of 50 of the 100 corvids (50.0%, 95% CI: 39.9-60.1). With the exception of ravens (only one of which was examined in 1995-1996), all species of corvid examined were carrying *Syngamus*, but only rooks and carrion crows had in excess of ten pairs. Nineteen immature rooks (95.0%, 95% CI: 73.1-99.7) examined in April to June had burdens of *Syngamus* - in 15 of the 19 birds there were over ten pairs of *Syngamus*, six had over 50 pairs, and the maximum recorded in one bird was 121 pairs. Gapeworms were also common in immature carrion crows (71.4%, 95% CI: 30.3-94.9), but unlike immature rooks none had over 50 pairs and the maximum number recorded in an immature carrion crow was 38 pairs.

Hairworms of the subfamily Capillariinae or their eggs were found in the oesophagus and/or oropharynx (and less commonly intestine) of 32 corvids (32.0%, 95% CI: 23.2-42.2). They were especially common in mature rooks (50.0%, 95% CI: 27.8-72.1), mature carrion crows (52.6%, 95% CI: 29.5-74.8), mature hooded crows (40.0%, 95% CI: 7.3-83.0) and mature jackdaws (37.5%, 95% CI: 10.2-74.1), but were also found in immature rooks and crows.

Tapeworms were found in 37 birds (37.0%, 95% CI: 27.7-47.3) - 22 rooks, ten carrion crows, three jackdaws, one magpie and one hooded crow. Seven tapeworms which were further examined by Mrs Eileen Harris at the Natural History Museum, London, belonged to the family Dilepididae, and one (from a carrion crow) was identified as *Dilepis undula*. Tapeworms were commonest in rooks, being found in 85.0% (95% CI: 61.1-96.0) of immature rooks in April to June, 30.8% (95% CI: 10.4-61.1) of mature rooks in April to June, and in 14.3% (95% CI: 0.7-58.0) of rooks submitted out-with these three months. Tapeworms were also common in carrion crows, seen in 60.0% (95% CI: 17.0-92.7) of mature birds in April to June, 42.9% (95% CI: 11.8-79.8) of immature birds in April to June, and 28.6% (95% CI: 9.6-58.0) of carrion crows submitted out-with these months. Small numbers of roundworms subsequently identified as *P. ensicaudatum* were present in the intestine of two carrion crows and one hooded crow, a few unidentified thorny-headed worms (acanthocephala) were found in a carrion crow and magpie, and *Cyathostoma* sp. syngamid worms were detected in the oropharynx of one bird, a hooded crow.

During the full twenty years of the study, significant parasitic burdens, often mixed, were found in 37 rooks, 13 carrion crows, one hooded crow, three jackdaws and five choughs. Gapeworms (Images 308-309, 312, 319-323), hairworms (Images 324-329, 379-380), gizzard worms (Images 388-403), thorny-headed worms (Images 357-361, 409-418) and intestinal flukes were associated with adverse effects on health in some birds, but tapeworms (Images 346-348, 356), *Porrocaecum* sp. roundworms (Image 339) and *Cyathostoma* sp. (Images 310-312) were incidental findings.

Many of the affected rooks were immature birds in poor body condition that had died or been culled because of ill-thrift, leg deformities and poor feathering. The tracheas of these birds were often partially occluded by gapeworms, with a blood-stained exudate in the lumen. Large numbers of tapeworms were also frequently present, and sometimes other internal parasites such as hairworms and coccidia were noted. Malnutrition may have been the primary problem in these immature rooks but the parasites, especially the gapeworms, were undoubtedly a contributory factor. Significant numbers of gapeworms were also found in some adult rooks. In some immature and adult rooks, heavy burdens of hairworms in the upper digestive tract resulted in a necrotic stomatitis and/or oesophagitis. Less frequently, significant burdens of hairworms were found in the small intestine. Some immature and adult rooks that died from trauma had heavy burdens of gapeworms, which may have predisposed them to injuries from vehicles. Conversely, in other birds, conditions such as corvid respiratory syndrome, beak deformity, chronic arthritis and neoplasia may have helped to change a low level of parasitism into a significant problem. Nine rooks with significant helminthosis also had heavy burdens of lice or *Ornithonyssus* sp. blood-sucking mites.

Significant numbers of gapeworms were detected in immature and mature carrion crows, but burdens tended to be lower than in rooks, with only one bird having over 50 pairs (52 pairs, in June 2002, therefore not included in Table 9.5). Stomatitis or oesophagitis caused by hairworms was diagnosed in immature and mature carrion crows. *Porrocaecum* sp. tended to be seen as part of a mixed parasitic infection in carrion crows more often than in rooks, but in no bird was the burden of *Porrocaecum* high enough on its own to be considered significant. Some crows with significant helminthosis had other conditions such as an abnormal beak, cellulitis, hypopyon and pneumonia. One hooded crow had a purulent stomatitis associated with many hairworms, and one malnourished carrion crow fledgling had many unidentified intestinal flukes and necrotic tracks in the liver from which *P. multocida* was isolated.

Significant helminthosis was not diagnosed in any magpies or ravens during this study, nor in jackdaws in 1995-1996. However in May/June 2002 large numbers (up to 42 pairs) of gapeworms caused the deaths of an adult jackdaw and two immature jackdaws.

Five choughs had burdens of internal parasites that were pathologically significant. Chough 1, a thin bird found in October, had a severe necrotic stomatitis (Images 379-380) associated with large numbers of hairworms, plus four pairs of gapeworms in the trachea. Chough 2, a current-year bird found in November, showed advanced autolysis but had a defect approximately 1 cm in diameter in the koilin layer of the gizzard. The surrounding koilin was black and ragged-edged, and the exposed gizzard mucosa was blackened and roughened. Many nematodes up to 16 mm in length and 1 mm in diameter were attached to or within the koilin layer, or on the exposed gizzard mucosa (Images 388-391). Small numbers of gapeworms were also present in the trachea. Microscopy confirmed that the nematodes in the gizzard belonged to the order Spirurida but were too degenerate for further identification. The worms did not belong to the genus *Streptocara*, because no cephalic collarette was present, nor the genera *Echinuria*, *Acuaria*, *Dispharynx* or *Cosmocephalus*, because no obvious cordons were apparent (Images 394-403). Similar spirurid worms were found in Chough 3, a four-year-old bird found dead in April, again associated with blackening and roughening of the koilin layer of the gizzard (Images 392-393). Most of the gizzard nematodes were found within or on the underside of the koilin. Small numbers of gapeworms, tapeworms and a single thorny-headed worm were also present. Gapeworms (at least 10 pairs in the trachea), many hairworms in the oropharynx and oesophagus, and many intestinal thorny-headed worms measuring up to 12 mm in length (Images 409-411) were detected in Chough 4, a thin one-year-old bird found dead in May. Large numbers of feather mites and lice were also present. Chough 5, a thin current-year bird found in August, had a haemorrhagic enteritis associated with thorny-headed worms up to 6 mm long, some of which were embedded in the mucosa of the terminal small intestine and rectum (Images 412-418). This bird also had a few pairs of gapeworms and moderate numbers of feather mites. The thorny-headed worms from Choughs 4 and 5 were identified by Mrs Eileen Harris

at The Natural History Museum, London, as *Plagiorhynchus* (*Prosthorrhynchus*) *cylindraceus*, a species which uses isopods such as woodlice (*Armadillidium vulgare*) as intermediate hosts.

Small to large numbers of coccidial oocysts (Images 344 a-f) were frequently detected in the intestinal contents of corvids but were not thought to be significant. When more detailed examinations were conducted between 1995 and 1996, coccidial oocysts were detected in 16 out of 100 corvids; five mature rooks, four mature magpies, three mature carrion crows, one mature jackdaw and three immature rooks. Oocysts from several birds were sporulated and had the appearance of isosporoid coccidia containing two sporocysts (Images 345 a-c). Further details of the helminths and oocysts described above can be found in Appendix III.

9.3.6 Corvid respiratory syndrome (CRS) and respiratory conditions not otherwise specified

Airsacculitis and/or pneumonia were seen in jackdaws with mycotic infections (see 9.3.4 above) and in some corvids with significant burdens of gapeworms (see 9.3.5 above). However, respiratory tract disease was seen in many other corvids. Twenty birds (13 rooks, six carrion crows and one jackdaw) were diagnosed as having “corvid respiratory syndrome (CRS)” and fulfilled the following diagnostic criteria: “*One or more of the following pathological features in corvids:- airsacculitis; tracheitis; pneumonia/pleurisy; sinusitis. Excludes a diagnosis of avian tuberculosis, mycotic pneumonia/airsacculitis or significant Syngamus burdens. May be concurrent chlamydiosis/chlamydiasis, mycoplasmosis or pasteurellosis. Lesions associated with large clusters of Gram-negative bacilli or coccobacilli on histopathology.*” Twenty-three other corvids (11 carrion crows, five rooks, three jackdaws, two hooded crows, one magpie and one raven) were coded as “Respiratory tract condition not otherwise specified (NOS)”, usually because no histopathology had been carried out or if Gram-negative bacteria were not detected on histopathology. Undoubtedly some of the “Respiratory tract condition NOS” cases would have been shown to be CRS if further

testing had been carried out. Images of CRS (Images 423-456) and respiratory tract condition NOS (Images 457-469) can be found in Appendix X.

The twenty birds diagnosed as having CRS originated from eleven different locations, nine in the south of Scotland and two in the north, and were submitted in January to March (9 birds), April to June (1 bird), July to September (5 birds) and October to December (5 birds). The commonest findings at necropsy were pneumonia/pleurisy (all 20 birds), airsacculitis (14 birds), pericarditis (11 birds) and sinusitis/nasal/ocular discharge (8 birds). Some birds also had enlargement of the liver or perihepatitis, and a necrotic stomatitis or oesophagitis associated with hairworms was present in some birds. Histopathology typically showed an acute fibrino-granulocytic exudative reaction in the lungs and airsacs, with areas of more chronic granulomata formation in some birds, associated with large clusters of Gram-negative bacteria.

The microbiological findings are summarised in Table 9.6. Bacteriology usually demonstrated mixed growths of a range of organisms including *Pasteurella*-like species (excluding *P. multocida*) and members of the Enterobacteriaceae. *P. multocida* was isolated from two birds, an adult carrion crow and an immature rook submitted from the same location approximately 18 months apart. Retrospective PCR testing of archived material detected *P. multocida* in pooled lung/trachea from all 10 birds tested, and immunohistochemistry (IHC) for *P. multocida* detected specific labelling in the lung of three out of four birds tested. Fungal cultures were carried out on tissues from 18 birds, but the only significant organism isolated was a small growth of *Candida albicans* from the lung and oropharynx of one bird with concurrent stomatitis.

Table 9.6: Corvid Respiratory Syndrome: microbiology

Test	Tissues	Number tested	Results
Standard bacteriology	Lung	20	<i>Pasteurella</i> -like organisms and/or Enterobacteriaceae: 18 birds <i>P. multocida</i> : 2 birds
Fungal cultures	Lung	18	<i>C. albicans</i> : 1 bird
PCR for <i>P. multocida</i>	Pooled lung/trachea	10	All positive
IHC for <i>P. multocida</i>	Fixed respiratory tissues	4	Positive labelling in lung of 3 birds
PCR for <i>Coxiella burnetii</i>	Pooled lung/trachea	6	All negative
PCR for <i>Ornithobacterium rhinotracheale</i>	Pooled lung/trachea	6	All negative
PCR for <i>Avibacterium paragallinarum</i>	Pooled lung/trachea	6	All negative
PCR for avian pneumovirus	Pooled lung/trachea	6	All negative
PCR for avian influenza virus	Pooled lung, trachea, and other viscera	11	All negative
PCR for <i>C. psittaci</i>	Pooled liver, lung, intestine	16	Positive in 8 birds, negative in 8 birds
IHC for <i>C. psittaci</i>	Fixed respiratory tissues	4	Positive labelling in lung of 1 bird
PCR for <i>M. gallisepticum</i>	Conjunctival swabs and/or oropharyngeal swabs or lung	14	Strong positive: 11 birds Weak or very weak reactions (considered negative): 3 birds
Culture for mycoplasmas	Conjunctival swabs and/or oropharyngeal swabs or lung	9	<i>M. gallisepticum</i> : no birds <i>M. sturni</i> : 2 birds

Because of the consistent presence of large clusters of Gram-negative bacteria, PCR testing for *Coxiella burnetii*, *Ornithobacterium rhinotracheale* and *Avibacterium paragallinarum* was carried out on respiratory tissues from six birds, but none of the samples was PCR-positive. Tissues from the same six birds were tested by PCR for avian pneumovirus, again with negative results, as were tissues from 11 birds screened for avian influenza virus. Tissues (usually pooled liver, lung and intestine) from 16 birds were screened for *Chlamydia* spp. by PCR, and eight birds from six locations were

Chlamydia-positive; four immature rooks, two adult rooks and two adult carrion crows. However, when IHC was carried out on fixed tissues from four birds, *Chlamydia*-specific labelling was only detected in the lung of one bird. A PCR for *Mycoplasma gallisepticum* was carried out on swabs or tissues from 14 birds; samples from 11 birds (six locations) were strongly positive, and three gave weak or very weak reactions that were considered negative. Cultures for mycoplasmas were carried out on samples from nine birds, but no significant mycoplasmas were isolated. Further details about the screening for mycoplasmas in corvids are given in 9.3.7 below.

“Respiratory tract condition NOS” was diagnosed in 19 large corvids (carrion crows, hooded crows, rooks, raven) and four small corvids (jackdaws and magpie). Mixed bacteria were recovered from the respiratory tracts of these birds and *C. albicans* was isolated from three birds, but further testing was limited. The postmortem findings in the large corvids were similar to those found in birds with CRS (16/19 pneumonia/pleurisy, 11/19 airsacculitis, 6/19 pericarditis, 3/19 sinusitis). However, none of the four small corvids had airsacculitis or pericarditis.

9.3.7. Screening for *Mycoplasma* spp.

Respiratory tract tissues or swabs were collected from 54 corvids, including nine choughs, and screening for mycoplasmas was conducted at Liverpool University. A PCR for *M. gallisepticum* was carried out on material from all 54 birds, and mycoplasma cultures were attempted in 41 birds. *M. sturni* was isolated from 11 birds (six rooks, three carrion crows and two magpies). Most of the rooks and crows with *M. sturni* were malnourished fledglings, often with significant helminthosis, and the two magpies had encephalitis and trauma respectively. *M. gallisepticum* was isolated from conjunctival swabs from two fledgling choughs with eye abnormalities and from an oropharyngeal/tracheal swab from one of the birds. Using DNA fingerprints generated by Random Amplified Polymorphic DNA (RAPD) analysis, the isolates from the two birds appeared identical to each other and to a pheasant (*Phasianus colchicus*) isolate,

but could be readily distinguished from a UK chicken isolate, a UK turkey isolate and a vaccine strain of *M. gallisepticum*.

Of the 54 corvids screened for *M. gallisepticum* by PCR, 14 gave positive results and another five were weakly positive or very weakly positive. The 14 birds that were positive comprised two choughs (also *M. gallisepticum* culture-positive), three carrion crows (two with CRS as in 9.3.6 above) and nine rooks (all with CRS). None of the magpies or jackdaws tested gave convincing PCR-positive results for *M. gallisepticum*. The results for the 38 rooks and carrion crows tested are summarised in Table 9.7; 85% of those with CRS were PCR-positive, but only 4% of those without lesions of CRS were PCR-positive. The difference in PCR-positivity between rooks/carrion crows with and without CRS was highly significant ($p < 0.001$, two-tailed Fisher's exact test).

Table 9.7: PCR for *M. gallisepticum* in rooks and carrion crows (n=38).

Status*	Number of birds
PCR positive, CRS at necropsy	11
PCR positive, no CRS at necropsy	1
PCR negative, CRS at necropsy	2
PCR negative, no CRS at necropsy	24

* weak or very weak PCR reactions were considered negative

9.3.8 Chlamydiosis/chlamydiasis and *Pasteurella multocida* infection

In addition to the eight birds (six rooks and two carrion crows) with CRS (9.3.6 above), *C. psittaci* was demonstrated by PCR in a malnourished jackdaw fledgling with fluid intestinal contents. *P. multocida* was isolated from four birds; from lungs and airsacs of a rook and a carrion crow with CRS (9.3.6 above), from the necrotic liver of a carrion crow with intestinal flukes, and from a caseous mass in the oropharynx of a rook with a severe beak deformity. The two isolates from birds with CRS were subsequently shown to be capsular type A.

9.3.9 Avian pox and other skin conditions

Four birds (three magpies and a jackdaw) had lesions typical of avian pox. An adult jackdaw had multiple nodules 1-3 mm in diameter around the eyes and under the mandible (Images 470-471), and three adult magpies had nodules on the toes (Images 472-473). Histopathology confirmed the diagnosis in two magpies. Two immature jackdaws had substantial loss of feathers of unknown cause over the breast and thighs (Images 474-475), and an immature magpie that died from necrotic enteritis had severe feather loss from the inner and outer aspects of both thighs (Images 476). Scaly leg (cnemidocoptic mange) was confirmed by demonstration of *Cnemidocoptes* sp. mites (Images 482-484) in three rooks, one carrion crow and one jackdaw. In the rooks and jackdaw, white or grey debris was present on the hocks, shanks or feet (Images 477-480), but the lesions in the carrion crow were more proliferative in nature, with raised corrugated thickening of the epidermis of the legs and feet (Image 481). Significant numbers of *Ornithonyssus* sp. mites (Images 485-490) were noted in 15 rooks and three carrion crows, and large numbers of lice (Images 491-500) were recorded in eight rooks and one hooded crow; all had other primary conditions such as respiratory disease, helminthosis, malnutrition and neoplasia. A chough with helminthosis also had large numbers of lice and feather mites, and moderate numbers of feather mites were present in another chough with helminthosis (Images 501-504).

9.3.10 Developmental abnormalities of the eyes and/or brain of young choughs

Between 2005 and 2013, fourteen chough nestlings from Islay, aged approximately 4-5 weeks, were euthanased on welfare grounds due to corneal opacity and submitted for necropsy. Postmortem examinations showed bilateral corneal opacity (Images 505-511), sometimes with dried discharges around the eyes and occasionally with disparity in the size of the globes (Images 513-518). In two birds the cornea was discoloured green (Image 512). In addition, five birds had hydrocephalus (Images 523-528), with excess fluid between the dura mater and the brain tissue, and abnormal doming of the skull was seen in one bird with hydrocephalus. Histopathology of the eyes of four affected birds

was carried out by Dr John Mould, Eye Veterinary Clinic, Leominster, and revealed combinations of the following features:

- Irregularity of the corneal epithelium, with an abnormal maturation sequence and inter- or intra-cellular vacuolation ([Images 519-522](#)). Round or polygonal cells were present in the surface layers rather than squames, and intra-epithelial vacuoles were apparent. In one bird there was ulceration and perforation of the central cornea.
- Thinning of the corneal epithelium axially but not near the limbus.
- Disorganisation of the corneal stroma, which was very cellular with cells orientated in random directions. Corneal oedema was apparent.
- Adherence of the lens to the cornea and/or iris.
- Adherence of the iris to the deep cornea.
- Multiple small vacuoles in the lens, with cataract formation.

These changes represented corneal dysgenesis associated with variable absence of Descemet's membrane and/or endothelium; multifocal iris-corneal adhesions; keratolenticular dysplasia; disorganisation and hypercellularity of the corneal stroma with increased matrix deposition; anterior cortical cataract; and corneal oedema and epithelial dysplasia.

Histopathology of the brain of four affected birds was carried out by Dr Jill Thomson of SACCVS and revealed the following features:

- Incomplete development of the cerebral cortex in all four birds. The normal layers of cortical grey matter were not apparent, and the cerebral hemispheres mainly comprised myelinated fibres and axons of the deep cortex.
- Normal thin pia mater but a thick dura mater with layers of fibrous tissue.
- Absence of inflammatory changes or abnormalities of the optic lobe, cerebellum or midbrain.

The eye abnormalities found in the choughs were similar to those observed in humans with Peters' anomaly, in which corneal opacity, adhesions between the cornea and iris, and an absence of Descemet's membrane are notable features (Traboulsi and Maumenee, 1992; Ozeki et al., 2000).

9.3.11 Necrotic enteritis and digestive tract conditions not otherwise specified

An immature magpie with feather loss from the legs had a large core of necrotic debris adherent to the mucosa of the duodenum, and haemorrhages in the mucosa of the lower intestine. Histopathology showed a severe segmental necrotic enteritis. Heavy mixed growths of *Clostridium perfringens* and *Fusobacterium necrophorum* were isolated from the intestine. The proventriculus was also thickened and *Candida albicans* was isolated from the proventriculus mucosa. In retrospect, this case showed similarities to the reovirus-associated necrotic enteritis described in corvids in Canada and the USA (Campbell et al., 2004; Meteyer et al., 2009), but virus isolation was not attempted.

Necrotic oesophagitis of unknown cause was found in five chough nestlings, two adult rooks and an adult carrion crow. In May 2012, a brood of four chough nestlings were found dead in their nest and the carcasses frozen. Two were in good body condition, one was small, and the fourth bird was small and autolytic. All four birds had a necrotic oesophagitis affecting the distal oesophagus near the thoracic inlet (Images 530-532, 534-541). The oesophageal mucosa was orange/yellow/brown, thickened and diffusely necrotic, extending through the oesophageal wall to the serosal surface where yellow-tinged serositis was evident. In the two birds in good condition, the oesophagus proximal to the affected area contained many food items (beetles and blowfly larvae) (Images 529, 533), and in one of the smaller birds a little food was found in the upper oesophagus. No food was present in the gizzard of any of the birds and the intestinal contents were sparse and dark. Wet preparations from the affected tissues and from the intestine did not detect any worm eggs or protozoa, and aerobic and anaerobic cultures did not demonstrate any significant bacteria, yeasts or fungi. Histopathological examination was impaired by the process of freezing and thawing but revealed severe

mucosal necrosis and inflammation of the oesophagus of the three birds examined, extending into the submucosa and associated with many bacterial colonies. Also in May 2012, the partially desiccated carcass of another chough nestling was found beneath a nest at another site and the carcass frozen. Despite the partial desiccation, freezing and thawing of the carcass, it was evident that the mucosa of the distal oesophagus was thickened and necrotic, similar to that of the choughs at the first site. The gross appearance of the oesophagus of these five birds was similar to that observed in some wild finches with trichomonosis (see Chapter 6) but a PCR for *Trichomonas gallinae* carried out on the oesophagus from three birds gave negative results, and trichomonads were not observed on the histopathological examination of the oesophagus of three birds, although interpretation was hampered by the freezing and thawing process.

Severe necrotic oesophagitis causing partial occlusion of the upper digestive tract was also diagnosed in two adult rooks and an adult carrion crow in unrelated sporadic incidents, and similar to the nestling choughs no significant bacteria, yeasts or helminths were detected. Multiple small white nodules (Images 547-548) were present in the oesophagus of a carrion crow found hanging in the branches of a tree; moderate numbers of hairworm eggs were present in the oropharynx, but no significant bacteria or yeasts were isolated from the oesophagus, and histopathology showed multiple heterophilic granulomata of unknown cause.

Other digestive tract conditions seen in corvids included perforation of the gizzard of a magpie by a piece of bone; ulcerative and granulomatous enteritis and liver necrosis in a jackdaw; and impaction of the gizzard and intestine of a fledgling jackdaw found under a garden feeder (Images 542-544). A carrion crow had massive liver necrosis and another carrion crow had a large cystic mass involving much of the liver (Images 545-546). The possible role of a reovirus in the cases of liver necrosis in the jackdaw and carrion crow, as reported by Lawson et al. (2015a) in a magpie, was not considered at the time.

9.3.12 Infection with *Salmonella* Typhimurium and *Escherichia albertii*

Tissues from 151 corvids were screened for the presence of salmonellae. *S. Typhimurium* was isolated from pooled tissues from two rooks through selenite enrichment; DT41 from a bird with respiratory disease and DT40 from a bird with bacterial meningitis. The DT41 isolate was considered to be incidental, but the significance of DT40 from pooled intestine and liver through selenite was unclear because bacteriology was not carried out on the brain of this bird. Liver and intestine from 129 corvids were screened for *E. albertii*, and potentially-pathogenic *E. albertii* with the API-20E profile of 4144102 or 5144102 were isolated from one chough and one jackdaw (Appendix VI). However neither of the isolates was seropositive for O86:K61 and they were considered non-pathogenic. *E. albertii* is discussed more fully in Chapter 5.

9.3.13 Miscellaneous conditions and pathogens

Adverse environmental conditions

A diagnosis of adverse environmental conditions was made in five nestling rooks and two nestling choughs. The five rooks were from several nests that had been blown down in high winds ([Image 549](#)), and the two choughs were found in or below their nest and appeared to have been chilled.

Central nervous system condition not otherwise specified

An immature magpie had a domed skull and internal hydrocephalus, probably indicating a congenital abnormality, with a superimposed non-suppurative encephalitis. Another immature magpie had a purulent encephalitis as an extension of a facial cellulitis, and in addition had large numbers of unidentified cryptosporidia in the bursa of Fabricius. A fibrinopurulent meningitis in an adult rook may have been the result of salmonellosis – *S. Typhimurium* DT40 was recovered from pooled tissues through enrichment but brain was not included in the cultured tissues because there was no history of nervous signs.

Reproductive tract disorders

Two corvids had egg yolk peritonitis. One was a chough found dead near its nest, and the other was a rook with concurrent corvid respiratory syndrome.

Yersinia pseudotuberculosis and Staphylococcus aureus

Y. pseudotuberculosis was isolated from three thin fledgling choughs from two different nests. One bird had multiple pale yellow or white nodules 1-3 mm in the liver, lung and spleen, and in one bird the liver appeared grossly normal but a multifocal necrotic hepatitis was observed on histopathology. The third bird was euthanased because it was thin and unable to use one of its wings; this bird had a purulent arthritis and tenosynovitis of one elbow joint, plus miliary foci in the liver and spleen (Images 550-552). *S. aureus* was isolated from only one corvid, from the swollen hock joint of a magpie.

Arthritis and cellulitis

Purulent or caseous arthritis and/or cellulitis affecting the tissues of the shoulder, elbow or hock joints were seen in three carrion crows, three rooks, a magpie, a jackdaw and a chough. *S. aureus* and *S. intermedius* were isolated from the magpie and jackdaw respectively and *Y. pseudotuberculosis* from the chough, but no significant bacteria were demonstrated in the carrion crows or rooks. Subcutaneous cellulitis of the head and neck was noted in three magpies (Image 557) and over the ventral abdomen of two rooks (Images 553-556), and another rook had an abscess in front of one eye (Images 558-559), but no significant bacteria were recovered from the infected tissues. In one of the magpies there was also a severe purulent encephalitis and mixed bacteria including *Pseudomonas aeruginosa* were isolated from the brain.

Beak deformities

An adult rook had an abnormally long and curved upper beak. The upper beak of an immature rook was also deformed, with lateral twisting so that the point of the beak re-entered the side of the oropharynx. A large caseous mass from which *Pasteurella*

multocida was isolated extended from the oropharynx to the globe of the eye of this bird. An immature carrion crow had a crossed beak, and in addition had a large abscess on the palate from which *Fusobacterium necrophorum* was isolated.

Neoplasia

Two thin adult rooks had neoplastic lesions. One had a pigmented fibrosarcoma, with a large black mass replacing part of a kidney and a smaller black nodule in the skin near the vent. A squamous cell carcinoma was diagnosed in the second bird, which had fibrous nodules up to 1 cm in diameter in the oesophagus and diffuse thickening of the mucosa around the larynx (Images 560-562).

Poisoning

A diagnosis of accidental diazinon poisoning was made in an adult raven, one of two ravens found dead in good condition. Ewes and lambs had been gathered and dipped in a diazinon sheep dip in the locality, and the gizzard of the raven contained the scrotum and remnants of the tail of a lamb, plus an orange rubber ring previously applied to the lamb for the purpose of castration (Images 563-564). Residues of diazinon at a level considered to be lethal were demonstrated in the gizzard contents, and it was hypothesised that the scrotum and tail had fallen off the lamb after it had been dipped, and subsequently consumed by the raven. Many unhatched fly eggs were present on the feathers of the carcass (Image 565). Poisoning was also suspected in the other raven submitted at the same time but was not confirmed.

Secondary candidiasis

C. albicans was recovered from diseased tissues from five carrion crows, a magpie and a jackdaw. Three carrion crows with respiratory disease (one with CRS, two with respiratory tract condition NOS) had caseous debris in the oropharynx or infra-orbital sinus from which *C. albicans* was isolated, and the yeast was isolated from the lungs and airsacs of another two carrion crows with respiratory tract condition NOS and of a jackdaw with mycotic airsacculitis and pneumonia. The magpie differed from the other

corvids in that *Candida* was recovered from the thickened proventriculus of a bird with necrotic enteritis.

9.3.14 No diagnosis

No diagnosis was made in 32 corvids. Most were immature or adult birds in poor condition for which no cause was demonstrated. Some others had shown central nervous system signs immediately prior to death and trauma was suspected but could not be confirmed. Five chough nestlings were found dead in or near their nests on different occasions but the cause of death was not determined, and poisoning or trauma was suspected but not confirmed in three adult ravens found dead in good condition, two near their nest.

9.3.15 Incidental findings

Tissues from 93 birds were sent to AHVLA Weybridge to be screened for avian influenza virus, but no positive results were reported. Similarly, tissues from 105 birds were sent to AHVLA to be screened for West Nile virus, but no positive results were received.

9.4 Discussion

9.4.1 General comments

The commonest causes of death identified in corvids in this study were trauma and malnourishment of young fledglings. Significant helminthosis, a corvid respiratory syndrome, and eye/brain abnormalities of young choughs were also important causes of death or euthanasia and are discussed more fully below.

Mycotic pneumonia/airsacculitis and mycobacteriosis were confined to jackdaws, and avian pox was seen in magpies and a jackdaw, suggesting that small corvids (magpies and jackdaws) may suffer from a different range of conditions to large corvids (rooks and crows). In contrast, large numbers of *Ornithonyssus* sp. mites were seen on large corvids but not small corvids; the mites were assigned to the genus *Ornithonyssus* on the basis of the appearance of the anal plate (ovoid, with the anus at the anterior end) but the species of mite was not determined. This is believed to be the first report of *Ornithonyssus* sp. mites in UK corvids, but it is likely that those working in wildlife rehabilitation centres are well aware of its presence in large corvids (Stocker 2000). *O. sylviarum* (the northern fowl mite) has been described in rooks in west Siberia (Yakimenko et al., 1990), and if the mite found in UK corvids is also *O. sylviarum*, corvids could provide a reservoir of infection that could spread to poultry. Scaly leg mites (most likely *Cnemidocoptes jamaicensis* or *Cnemidocoptes intermedius*) were detected in large and small corvids, but are less likely to spread to poultry, in which the related *Cnemidocoptes mutans* is the cause of scaly leg (Mason and Fain, 1988; Dabert et al., 2011).

Relatively few corvids were screened for *C. psittaci*, but the demonstration by PCR of the organism in nine birds showed that this group of birds could pose a threat to the health of humans, especially those working at wildlife rehabilitation centres, and to other wild birds at these centres. These risks should be emphasised to rehabilitators and appropriate precautions taken. It would be useful to carry out a larger screening

programme to estimate the prevalence of chlamydiae in corvids, including screening for *C. suis* and determination of the genotypes of *C. psittaci* present.

Unlike the situation in small garden birds (Chapters 3-7), trichomonosis, salmonellosis and *E. albertii* bacteraemia were not confirmed in corvids, again highlighting differences in disease susceptibility between different groups of wild bird. Nevertheless, a severe necrotic oesophagitis similar to that seen in finches with trichomonosis was observed in some corvids, the aetiology of which was not determined. Investigations were limited because of the poor condition of the material submitted, but a PCR for *T. gallinae* was carried out on tissues from three birds with negative results. More detailed investigations should be carried out if further material becomes available in the future. The possible role of reoviruses in corvids, causing necrotic lesions in the liver, spleen or intestine as described by Lawson et al. (2015a), should also be explored in the future.

9.4.2 Significant helminthosis

The techniques used to detect helminths in this study were of a screening nature rather than an attempt to recover and quantify all the helminths of the respiratory and digestive tracts, and inevitably some birds with small numbers of parasites will not have been identified. The three helminth groups found most frequently were the gapeworm *S. trachea*, hairworms of the subfamily Capillariinae and tapeworms of the family Dilepididae. Earthworms can carry the larvae or intermediate stages of these three helminths (Pemberton 1963; Anderson 1992) and are likely to be the major route by which the corvids became infected.

Gapeworms were most prevalent in immature rooks in April to June, reflecting the importance of earthworms in the diet of nestling rooks as supplied by the parent birds. Provided they are accessible, 70-80% of the diet of young rooks is made up of earthworms, together with grain, leatherjackets, beetles, ants, spiders and flies (Coombs 1978). Many of the immature rooks with significant helminthosis were malnourished as described in 9.3.3, and the burdens of *Syngamus* would have added to their problems.

Earthworms are also the most important source of animal protein in the diet of adult rooks (Coombs 1978), explaining the presence of gapeworms in adult rooks in April to June. Available earthworm numbers decrease from June onwards, partly accounting for the fall in the percentage of adult rooks with gapeworms after June. The situation in carrion crows was similar, but the percentage of infected immature carrion crows was lower than in immature rooks, and the numbers of parasites tended to be much lower in the immature carrion crows. Again, this reflects the diet of carrion crows, which are very adaptable feeders and less dependent on earthworms than rooks, utilising a wide range of foodstuffs of which grains, wild plant seeds, carrion and live small mammals are especially important (Coombs 1978). Earthworms are also less important in the diets of hooded crows, magpies, jackdaws and ravens (Coombs 1978), and *Syngamus* burdens in such birds were usually small and not significant, with the exception of some jackdaws in 2002.

A similar pattern was seen with the detection of tapeworms, which were most prevalent in immature rooks but were also frequently found in immature carrion crows, mature carrion crows and mature rooks, especially in the months April to June. Although gapeworms and tapeworms were commonest in April to June, hairworms were more often seen in the remaining nine months of the year, the exception being rooks in which hairworms were also commonly seen in April to June. Hairworms were least commonly detected in immature birds, suggesting that, compared with gapeworms and tapeworms, there is a lower level of challenge or a greater time interval between ingestion of hairworm eggs or intermediate hosts and subsequent detection of hairworms in the birds.

This study has shown that many wild birds of the family Corvidae found by members of the general public and presented to wildlife rehabilitation centres are likely to be carrying heavy helminth burdens, especially immature rooks and carrion crows in April to June. It is well-recognised that the distribution of parasites within a host population is not uniform, with most hosts having relatively few parasites but a few members of the host population harbouring the majority of the total parasite population (Anderson and

May, 1978). Many of the corvids examined in this study belonged to the latter category, and are probably not representative of the overall corvid population. The ethical arguments for and against attempting to treat and rehabilitate wildlife casualties such as those encountered in the present study were discussed in Chapter 1. Kirkwood and Best (1998) considered that treatment was more justifiable if the wildlife injury or illness was the result of human actions, but that the treatment of wildlife that had become sick or injured through natural processes might be considered an inappropriate interference with nature, and that in some circumstances euthanasia at an early stage might be the best course of action. It could, therefore, be argued that this would be the most appropriate action to take in the case of immature rooks and carrion crows presented with evidence of malnutrition and with heavy parasite burdens. If, however, the treatment and rehabilitation of corvid casualties was to be undertaken, the results of this study suggest that rooks and crows should routinely receive an appropriate wormer at the start of the treatment and rehabilitation process.

The pathogenic effects of gapeworms in choughs have previously been recorded (Bignal et al., 1987; Meyer and Simpson, 1988) but in the current study adverse effects from hairworms, spirurid gizzard worms and thorny-headed worms were also encountered. The role of intermediate or transport (paratenic) hosts in the transmission of the hairworms encountered is unclear, but paratenic hosts such as earthworms, slugs, snails, beetles and some flies are known to be important in the transmission of the gapeworm *S. trachea* (Taylor et al., 2007). Similarly, amphipod crustaceans such as sandhoppers and isopod crustaceans such as woodlice are intermediate hosts for some spirurid worms and thorny-headed worms. The diet of choughs, and any change to the diet resulting from food item availability, could therefore have an impact on exposure to potential pathogens. In recent years, there has been a decline in the breeding population of choughs on Islay, largely due to a significant fall in first-year survival (the probability that a chough survives from fledging to one year of age), resulting in reduced recruitment into the breeding population (Reid et al., 2011). Analysis of re-sighting data of colour-ringed birds indicated that most of the additional mortality contributing to

reduced first-year survival occurred in the months July to November, a period when the young birds have left their natal territories and parental care to join larger flocks of sub-adult birds (Reid et al., 2011). It is possible that deaths from helminthosis are contributing to this fall in first-year survival of choughs. A high rate of inbreeding in this endangered population (see 9.4.4) may also have predisposed to helminthosis.

9.4.3 Corvid respiratory syndrome (CRS)

A diagnosis of CRS, as defined in 9.3.6 and Appendix II, was made in 20 corvids. All had pneumonia/pleurisy, 14 had airsacculitis (often severe), 11 had pericarditis and 8 had sinusitis or evidence of a nasal/ocular discharge. Histopathology revealed an acute fibrino-granulocytic pneumonia and airsacculitis, sometimes with areas of more chronic granulomatous pneumonia, associated with large clusters of Gram-negative bacilli or coccobacilli. Despite the consistent gross findings and histopathological features, bacteriology gave very mixed results and pathogens such as *P. multocida* were isolated from only two birds. PCR did, however, demonstrate *P. multocida* in pooled lung/trachea from all ten birds tested, and a PCR for *M. gallisepticum* was strongly positive in 11 of 14 birds tested. Similar to the situation with *P. multocida*, despite its frequent detection by PCR, *M. gallisepticum* was not cultured from any of the birds with CRS. In addition, *C. psittaci* was detected by PCR in 8 of 16 birds tested. Several different combinations of potential pathogens were therefore detected during this study, and the pathology observed was thought to represent a syndrome rather than one specific disease.

The gross and histopathological findings of CRS were very similar to those described in corvids by Strugnell et al. (2011), who were able to isolate *P. multocida* from affected birds received alive but not from those submitted dead. These authors were also able to demonstrate by immunohistochemistry the presence of *P. multocida* in affected lungs of all birds submitted alive and most submitted dead, and concluded that *P. multocida* was the cause of the disease. There is clearly a strong association between CRS and *P. multocida* detection, but it remains uncertain why the organism was not isolated more

frequently from birds with CRS; in other circumstances such as pasteurellosis in chickens and turkeys, and garden birds suffering from pasteurellosis following a cat attack, the organism can be readily cultured from dead birds. Further screening of healthy and diseased corvids by culture and by PCR would help shed light on this anomaly, and may reveal that healthy corvids carry *P. multocida* in the oropharynx, similar to the picture seen in other species of birds and mammals. Muhairwa et al. (2000) recovered *P. multocida* from 80% of healthy breeding chickens and 73% of healthy laying chickens from two flocks that had previously experienced deaths from *P. multocida*, and from 63% of healthy breeding Muscovy ducks and Pekin ducks. Rimler and Glisson (1997) concluded that healthy birds carrying the organism in the upper respiratory tract were the most likely source of infection in poultry, and noted that most species of farm animals could carry *P. multocida*. These authors also commented that isolates from mammals were not necessarily pathogenic to poultry, and Christensen et al. (2008) suggested that different subtypes of *P. multocida* were responsible for respiratory tract infections in different species of animal and bird.

A PCR for *M. gallisepticum* was strongly positive in 11 out of 13 rooks/carrion crows with CRS, and in contrast only 1 out of 25 rooks/carrion crows that did not have CRS was *M. gallisepticum* PCR-positive. This association between PCR positivity/negativity and the presence/absence of CRS was statistically highly significant ($p < 0.001$, two-tailed Fisher's exact test) suggesting that *M. gallisepticum* or a similar mycoplasma may be playing a role in this syndrome. The failure to culture *M. gallisepticum* despite the PCR being positive may reflect the fastidious nature of some species of mycoplasma and the level of specificity of the *M. gallisepticum* PCR, which also detects other species of mycoplasma such as *M. imitans*. It is possible that the PCR is detecting a mycoplasma of corvids other than *M. gallisepticum*, that does not grow under standard laboratory conditions. Interactions between mycoplasmas and bacteria are well-recognised in some farmed species. Damage to the upper respiratory tract caused by *M. gallisepticum* can allow *E. coli* to cause chronic respiratory disease in poultry (Barnes and Gross, 1997), with lesions very similar to those observed in CRS, and *M. hyopneumoniae* infection in

pigs predisposes to *P. multocida* infection of the lungs (Amass et al., 1994). A combination of *M. ovipneumoniae* and *Mannheimia* (previously *Pasteurella*) *haemolytica*, a bacterium found in the nasopharynx of healthy cattle and sheep, can result in an acute fibrinous pneumonia and pleurisy in sheep (Ayling and Nicholas, 2007), and in cattle *M. bovis*, *M. dispar*, *M. bovirhinis* and *Ureaplasma diversum* can predispose to pneumonia caused by *Mannheimia haemolytica* and *P. multocida* (Caswell and Williams, 2007; Nicholas 2011). It is therefore possible that CRS arises from an initial *M. gallisepticum*-like challenge, permitting secondary invasion by a strain of *P. multocida* carried in the upper respiratory tract of healthy corvids.

The role of *C. psittaci* in CRS is likewise unclear. The demonstration by PCR of the organism in 50% of cases of CRS tested may be an incidental finding, but it is possible that *C. psittaci* is exacerbating the effect of the other pathogens present and its role cannot be discounted. For example, respiratory disease in cats can result from a combined infection with *C. felis*, *Mycoplasma* spp. and bacteria such as *Bordetella bronchiseptica* (Barrs and Beatty, 2013). The PCR used in the corvids in the current study detected the outer membrane protein of *C. psittaci* (Paul Burr, personal communication), but in light of the detection of *C. suis* in 21 magpies and hooded crows in Italy by Di Francesco et al. (2015), further characterisation of chlamydiae from corvids with CRS should be carried out. It is interesting to note that the outbreak of CRS investigated by Strugnell et al. (2011) occurred on a pig farm, but testing for chlamydiae was not performed.

Other pathogens may also be contributing to CRS. Dahl et al. (2002) showed that chickens artificially infected with large roundworms (*Ascaridia galli*) and then inoculated with *P. multocida* were clinically more severely affected, had more pathological lesions, and excreted *P. multocida* for longer than birds with single infections. Mixed helminth burdens are very common in corvids (see 9.3.5 above) and although birds with heavy burdens of *S. trachea* were excluded from the diagnosis of

CRS, damage caused by migrating internal parasites may have contributed to the development of CRS in some birds.

In summary, CRS appears to be a multifactorial condition involving combinations of different organisms as discussed above. Further investigations by those conducting wild bird disease surveillance may help to elucidate the significance of the organisms detected and their potential for spread to humans and livestock. Those working in wildlife rehabilitation centres should also be aware of this condition, and if diagnosed in casualty birds, should take appropriate measures to prevent its spread to corvids or other groups of birds.

9.4.4 Developmental abnormalities of the eyes and/or brain of young choughs

Fourteen nestling or fledgling choughs were euthanased and submitted for necropsy because of unilateral or bilateral corneal opacity. Necropsy confirmed the gross findings, and in addition some birds had hydrocephalus. Histopathology subsequently showed that the eye abnormalities, including corneal opacity, adhesions between the cornea and iris, and an absence of Descemet's membrane, were similar to those observed in humans with Peters' anomaly (Traboulsi and Maumenee, 1992; Ozeki et al., 2000). Peters' anomaly in humans, a form of anterior segment dysgenesis, can also be accompanied by other ocular and non-ocular abnormalities such as adhesions between the cornea and lens, microphthalmia and defects of the central nervous system (Traboulsi and Maumenee, 1992; Ozeki et al., 2000), similar to those observed in some of the choughs in this study. The necropsy findings in the choughs were also similar to those described in two captive-bred snow leopards (*Panthera uncia*), in which corneal opacity, cataracts, microphthalmia and hydrocephalus were attributed to Peters' anomaly (Hamoudi et al., 2013).

Peters' anomaly in humans is thought to be the result of the sporadic or inherited mutation of one or more genes involved in the development of the anterior segment of the eye, and, depending on the genes involved, may be inherited in an autosomal

recessive or autosomal dominant pattern. The eye and brain lesions observed in the choughs from Islay were also suggestive of a developmental abnormality, most likely inherited, that would have caused the deaths of affected birds before or after fledging. Wenzel et al. (2012) highlighted the low genetic diversity within the chough population on Islay and raised concerns that this could adversely impact on bird fitness at an individual and population level. Woolliams (2012) noted that inherited diseases caused by recessive genes could become a problem if population size was small and the rate of inbreeding was high. The same author also stressed that the genetic influence on disease went much further than inherited conditions, but also applied to genetic variation in susceptibility to endemic diseases. In the case of the Islay choughs, the low genetic variation may have resulted in the emergence of a deleterious inherited defect affecting the development of the eye and brain. Supporting evidence for this hypothesis has recently been provided by Trask et al. (2016), who retrospectively examined the family records of affected choughs on Islay and showed that the distribution of blindness was consistent with that caused by an inherited single-locus autosomal lethal recessive allele. Awareness and greater understanding of this condition will influence future conservation strategies for this endangered population of choughs.

Chapter 10

Diseases of UK ‘other passerines’

10.1 Introduction

Birds of the world are classified into 28 orders, with the greatest number of species placed in the order Passeriformes (passerine or perching birds). Nearly 250 species of passerines have been recorded in the UK, including those in the Families Carduelidae, Fringillidae, Passeridae, Emberizidae, Prunellidae, Paridae and Aegithalidae (Chapters 3-7), Turdidae and Sturnidae (Chapter 8) and Corvidae (Chapter 9). Other important families of the order Passeriformes found in the UK are the Alaudidae (larks), Hirundinidae (swallows and martins), Sylviidae (warblers), Bombycillidae (waxwings), Sittidae (nuthatches), Certhiidae (treecreepers), Troglodytidae (wrens), Cinclidae (dippers) and Motacillidae (wagtails and pipits). Those families not previously considered in Chapters 3-9 will be referred to as ‘other passerines’ and are discussed in this chapter.

More than 6.5 million pairs of warblers and 1.3 million pairs of swallows (*Hirundo rustica*) and house martins (*Delichon urbicum*) breed in the UK (Musgrove et al., 2013), the vast majority then returning to various parts of Africa for winter. Small numbers of waxwings (up to 11,000 birds, Musgrove et al., 2013) may visit the UK in autumn and winter, depending on the availability of berries such as rowan, whitebeam and hawthorn. These birds probably come from Fennoscandia and northern Europe, but their precise origin is uncertain (Duncan 2002). Wrens are relatively sedentary and common, around 8.6 million breeding pairs being found in the UK (Musgrove et al., 2013). Goldcrests (*Regulus regulus*) are less common, with a UK breeding population of about 610,000 pairs (Musgrove et al., 2013). The UK breeding population of meadow pipits (*Anthus pratensis*) has been estimated to be around 1.9 million breeding birds, and although most spend the winter in the Iberian peninsula, they are replaced by passage migrants from Iceland, Norway and the Faeroe Islands (Dougall 2002a). Similarly, around 470,000 pairs of pied/white wagtails breed in the UK; some then winter in Spain, Portugal and

Brittany, to be replaced by passage migrants from Greenland, Iceland, Norway and the Faeroe Islands (Dougall 2002b; Musgrove et al., 2013). The commonest breeding lark in the UK is the skylark (*Alauda arvensis*) (1.5 million breeding pairs, Musgrove et al., 2013), with winter numbers increased by continental skylarks on passage or wintering in the UK (Gillings and Dougall, 2002). Nuthatches, treecreepers (*Certhia familiaris*) and dippers (*Cinclus cinclus*) are sedentary species, with UK breeding populations of around 220,000 pairs, 200,000 pairs and 18,000 pairs respectively (Musgrove et al., 2013). None of the species of ‘other passerines’ examined during the study was red-listed as being of conservation concern in the UK (Hayhow et al., 2014). The Latin names of the UK birds discussed in this chapter are listed in Appendix I.

10.2 Review of diseases found in UK ‘other passerines’

References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.

10.2.1 Trauma and pasteurellosis

Similar to the situation in the passerines discussed in Chapters 3-9, trauma is a common cause of death in ‘other passerines’. The different species accounts presented in the *Migration Atlas: movements of the birds of Britain and Ireland* (Marchant et al., 2002) frequently refer to deaths in ringed birds from collisions with vehicles or man-made structures, losses to natural and domestic predators, and deliberate taking by man (usually out-with the UK). Secondary pasteurellosis may occur in birds that survive an earlier cat attack (Macdonald et al., 1981; Stocker 2000).

Mass mortalities due to trauma have been reported in waxwings (e.g. Anon 2010d; Anon 2011c). Affected birds were found next to large buildings with many windows, and postmortem findings were consistent with window collisions.

Some ‘other passerines’ feed in and around airports, and between 2009 and 2012 swallows were the species most commonly identified as being involved in aircraft

strikes. During these years, house martins, skylarks and meadow pipits also frequently appeared in the top ten identified species (Civil Aviation Authority, 2012).

10.2.2 Poisoning

Accidental poisoning with yellow phosphorus (once used in some rat poisons) was reported in a wren (Jennings and Soulsby, 1956), and Jennings (1961) concluded that passerines (and owls) were especially at risk from poisoning by phosphorus compounds. Macdonald (1962b) listed poisoning by dieldrin or heptachlor seed dressing as the probable cause of death of skylarks from two locations. Although a problem in corvids (Chapter 9) and pigeons/doves (Chapter 11), abuse or misuse of pesticides, rodenticides and other products have not been major issues in ‘other passerines’ in recent years. Of 987 confirmed incidents of pesticide poisoning between 1998 and 2004, none involved ‘other passerines’ (WIIS 1998-2004). However Johnson (1996) made the point that poisoning in such small species was more likely to go unnoticed than in larger birds such as geese.

Deaths from trauma secondary to intoxication with ethanol or cyanide, acquired from feeding on berries prior to death, were suspected in waxwings (Anon 2010d; Anon 2011c). Poisoning was not confirmed in these cases, but suspected ethanol toxicity has been reported in blackbirds and a redwing in the UK (Duff et al., 2012), and trauma following ethanol toxicosis was diagnosed in cedar waxwings (*Bombycilla cedrorum*) in America (Fitzgerald et al., 1990; Kinde et al., 2012). Keymer (1958) noted that waxwings can consume the berries of yew trees without being poisoned.

10.2.3 Infection with *Salmonella* spp., *Campylobacter* spp. and *Yersinia pseudotuberculosis*

Salmonellosis is an important cause of mortality in finches and sparrows, and is discussed fully in Chapter 4. There have, however, been few reports of the isolation of *Salmonella* spp. from ‘other passerines’ in the UK. *Salmonella* Typhimurium was recovered from the carcase of a lesser whitethroat (*Sylvia curruca*) by Goodchild and

Tucker (1968) but not from a further 33 carcasses or cloacal swabs. Pennycott* et al. (2006) and Lawson et al. (2010) did not recover the organism from an unspecified number of carcasses of ‘other passerines’ screened between 1995 and 2003, and 73 faecal samples from captured ‘other passerines’ feeding at two sewage treatment works in England were negative for *Salmonella* spp. (Plant 1978).

Campylobacter jejuni, a significant cause of food-poisoning in humans, has frequently been isolated from starlings (Chapter 8), corvids (Chapter 9) and pigeons/doves (Chapter 11). No studies examining the carriage of campylobacters by UK ‘other passerines’ were found, but Waldenstrom et al. (2002) showed that the carriage of *Campylobacter* spp. in wild birds in Sweden varied depending on feeding ecology. *Campylobacter* carriage by aerial insectivores (e.g. house martins and swallows) and arboreal insectivores (e.g. warblers) was low, but was relatively high (20.3%) in ground foraging insectivorous birds such as wagtails and pipits. No conclusions were drawn as to the potential pathogenicity of the avian isolates for humans.

The house martin was second only to the woodpigeon on the list of wild birds from which *Yersinia pseudotuberculosis* was isolated between 1961 and 1971 by Mair (1973). Other birds included the swallow and pied wagtail, and Mair speculated that insectivorous birds may become infected by eating insects that have previously fed on faecal material from other wild birds and rodents. Zurek et al. (2001) demonstrated that house flies (*Musca domestica*) can carry *Y. pseudotuberculosis* for up to 36 hours after exposure. In addition, bites from insects and ticks may be involved in the transmission of this organism (Mair 1973). Pseudotuberculosis (yersiniosis) in a house martin and swallow was also diagnosed by Macdonald (1962b), in a willow warbler by Jennings and Soulsby (1957), in skylarks (Clapham 1957; Jennings 1959) and in a wren (McDiarmid 1956).

10.2.4 Ticks, other external parasites, tick-borne spirochaetes, and haematozoa

In a study to determine the role played by passerine birds in the transmission of the tick-borne spirochaete *Borrelia burgdorferi* sensu lato, the cause of Lyme disease in humans, James et al. (2011) examined 1229 birds, including 25 ‘other passerines’, for the presence of ticks. No adult or nymphal ticks were detected in these ‘other passerines’, but 12 birds (five wrens, one blackcap (*Sylvia atricapilla*), four willow warblers and two treecreepers) had tick larvae. All the ticks examined were identified as *Ixodes ricinus*. No further testing was carried out on the larval ticks from these ‘other passerines’ and the study showed that blackbirds and thrushes were more likely to be important in the transmission of *B. burgdorferi* to humans (see 8.2.8 of this thesis). Other studies, however, have reported both larval and nymphal stages of *I. ricinus* on ‘other passerines’ and demonstrated the causal organism of Lyme disease in the larvae or nymphs. Using PCR technology, Olsen et al. (1995) detected *Borrelia* DNA in the larvae or nymphs of *I. ricinus* from pipits, wrens and warblers captured in Sweden and Denmark, and concluded that several species of passerines could act as reservoirs of *B. burgdorferi*. The same is likely to be true in the UK. Other species of ticks may also be detected in ‘other passerines’ in the UK; *I. lividus* was detected in sand martins (*Riparia riparia*), and *I. frontalis* in warblers and wrens, by Pietzsch et al. (2008).

The commonest ectoparasites found on house martins and swallows, or in their nests, are hippoboscids (also known as louse flies or flat flies) and fleas. Hippoboscids such as *Crataerina hirundinis* are flattened dorso-ventrally (hence the alternative names of louse flies or flat flies), have large claws, and long but very narrow wings. Larvae develop inside the females, and when deposited in the nest or feathers immediately start to pupate, forming shiny brown or black puparia which look like hard seeds. The examination of one house martin nest in England revealed over 5,000 fleas (mostly *Ceratophyllus hirundinis* and *C. rusticus*) and over 150 puparia of the hippoboscids fly *Crataerina hirundinis* (George 1989). In addition, there were 80 adult or nymph stages of the martin bug (*Oeciacus hirundinis*), which is related to the human bed-bug and can occasionally invade houses and bite humans. The same author reported over 1,000 fleas

(*Ceratophyllus styx*) in the nest of a sand martin. Hippoboscids found in swallows are likely to be *Ornithomya biloba* or *Crataerina hirundinis*, and the latter has also been found in sand martins (Hutson 1984). Fleas, martin bugs and hippoboscids are all blood-suckers and may have a direct adverse effect on their host, and hippoboscids in the UK may also be involved in the transmission of bacteria and the blood protozoan *Haemoproteus* spp. (Hutson 1984). A study carried out in The Netherlands showed that 70% of adult house martins in summer were infected with *Haemoproteus* sp. and 9% carried *Plasmodium* sp. (Piersma and van der Velde, 2012). None of the current year fledglings were positive for these haemoparasites, and these authors speculated that infections were acquired at the wintering grounds. A similar study carried out in Spain (Marzal et al., 2005) reported that 71% of adult house martins tested in the breeding season were positive for haemoparasites. Although the Spanish study found a negative association between haemoparasite infection and body mass and local survival, such a relationship was not found in the study in The Netherlands.

10.2.5 Miscellaneous conditions and pathogens

Adverse environmental conditions

Some species of ‘other passerines’ can be severely affected by prolonged periods of cold weather. Following the severe winter of 1962-1963, the breeding population of wrens was reduced to less than a quarter of the previous year, and for skylarks, meadow pipits and pied wagtails the breeding population fell by more than 50% (Dobinson and Richards, 1964). These four species were in the top six species most affected by the adverse weather, the other two species being the mistle thrush and song thrush. Peach et al. (1995) noted that wrens and treecreepers suffered very high mortality in the winters of 1978-1979 and 1985-1986, but for different reasons; wren deaths were linked to the number of snow days, but treecreeper deaths were associated with the amount of rainfall. Birds such as swallows, skylarks and meadow pipits may be found dead after becoming trapped inside buildings while prospecting for food or nesting sites (Marchant et al., 2002).

Cnemidocoptic mange (scaly leg)

Infection of the feet and lower legs with the burrowing mite *Cnemidocoptes* sp. (scaly leg) was described in chaffinches (Chapter 3) and corvids (Chapter 9). This disease has also been suspected or confirmed in sedge warblers (*Acrocephalus schoenobaenus*) in the UK and in their wintering grounds in Africa (Blackmore and Keymer, 1969; Fry et al., 1969; Thorne 1971). Affected birds had yellow crusting and distortion of the scales of the postero-lateral aspects of the legs. Similar lesions continue to be noted on-line by those viewing and ringing sedge warblers, for example the image and text circulated on BirdForum in 2013 (<http://www.birdforum.net/showthread.php?t=258448>). Accessed 01/02/16).

Mycotic pneumonia

Aspergillosis was diagnosed by Keymer (1958) in a waxwing found dead in a poultry run, but no further details were given.

Internal parasites – helminths and coccidia

There are few reports of helminths or coccidia from ‘other passerines’ in the UK. Clapham (1957) identified thorny-headed worms and trematodes in a wren that died from starvation, and Keymer et al. (1962) detected *Isospora* sp. coccidia in a whitethroat (*Sylvia communis*) as an incidental finding.

Listeriosis

Infection with *Listeria monocytogenes* was considered by Jennings (1955) to be the cause of death of a brood of three young whitethroats, but no further information was provided.

10.3 Diseases of ‘other passerines’ examined at Ayr DSC 1994 to 2013 – results

10.3.1 Bird species, numbers and locations

Forty-nine ‘other passerines’ were examined between January 1994 and December 2013. Of those for which a location was given, 79% were found in the south of Scotland and 21% in the north. A summary of the species examined and the conditions diagnosed can be found in Tables 10.1 and 10.2. The materials and methods are described in Chapter 2, and the diagnostic criteria used are listed in Appendix II.

Table 10.1: ‘Other passerines’ examined.

Family	Number examined
Hirundidae (house martins and swallows)	15
Sylviidae (blackcaps, warblers and goldcrests)	9
Bombycillidae (waxwings)	9
Motacillidae (meadow pipits, pied wagtails, grey wagtails)	7
Troglodytidae (wrens)	4
Certhiidae (tree creepers)	3
Cinclidae (dippers)	1
Sittidae (nuthatches)	1
Total	49

Table 10.2: Conditions* diagnosed in ‘other passerines’ (n=49).

Condition	Number of birds	% (95% confidence intervals)
Trauma	24	49.0 (34.6-63.5)
Adverse environmental conditions	9	18.4 (9.2-32.5)
Infection with <i>Yersinia pseudotuberculosis</i>	3	6.1 (1.6-17.9)
Miscellaneous conditions and pathogens:		All <3%
Mycotic pneumonia/airsacculitis	1	
Coccidiosis	1	
No diagnosis	10	20.4 (10.7-34.8)
Incidental findings:		Not applicable
Avian gastric yeasts in a waxwing	1	
Intestinal flukes in house martins, a swallow and a pied wagtail	6	
<i>Diplotriaena tridens</i> in airsacs of a blackcap	1	
Tissues screened for avian influenza virus – no positive results	19	
Tissues screened for West Nile virus – no positive results	11	

*Not all birds were examined for all conditions, and some birds had multiple conditions

10.3.2 Trauma and pasteurellosis

The commonest condition seen in ‘other passerines’ was trauma, diagnosed in almost half of the birds submitted. Six waxwings, submitted in November of two different years, had blood in the oropharynx, trachea, lungs, chest or abdomen. The birds were in good body condition and had unidentified berries in the upper digestive tract, proventriculus or gizzard ([Image 567](#)). The livers were often pale ([Image 566](#)) and in some birds clearly had ruptured. Three birds submitted as one batch were known to have collided with windows, and window collision was suspected in the second batch of three birds. Trauma was also diagnosed in another waxwing which was thin and had a ruptured crop. Wet preparations from the proventriculus of this bird demonstrated many avian gastric yeasts (“megabacteria”) measuring a mean of 34.8 µm x 2.4 µm, of uncertain significance. Such “megabacteria” were not detected in three waxwings that died after colliding with windows or in a waxwing with mycotic pneumonia. Window

collision was also strongly suspected in two meadow pipits, a blackcap, a treecreeper, a nuthatch and two swallows found under windows, and two other swallows were known to have been involved in aircraft strikes. Injuries caused by predators were seen in a grey wagtail (*Motacilla cinerea*) and an unidentified warbler, and shoulder injuries of unknown cause were present in two house martins. Trauma to the head, neck or wing was noted in two chiffchaffs (*Phylloscopus collybita*) and a willow warbler (*P. trochilus*), and one fat blackcap had extensive haemorrhages into the chest and lungs; this bird also had ten long thin worms measuring up to 45 mm in the thoracic airsacs (Images 574-575), subsequently identified by Mrs Eileen Harris of the Natural History Museum, London, as *Diplotrriaena tridens*. Bacteriology was carried out on tissues from 17 'other passerines' that had died from trauma, but *Pasteurella multocida* was not isolated from any of the birds.

10.3.3 Adverse environmental conditions

Four dead wrens were found during spells of very cold weather, a treecreeper was found dead during wet weather, and three pied wagtails were submitted after becoming trapped inside a school building over a weekend. A nestling house martin was found dead under its nest after house sparrows had evicted the parents and nestlings. Postmortem findings in these birds were often non-specific.

10.3.4 Infection with *Yersinia pseudotuberculosis*

Pseudotuberculosis was diagnosed in three house martins (Images 568-571). One bird had small white foci in the liver and spleen, and yellow plaques in the oropharynx. A second bird had necrotic foci 1-5 mm on the liver, and a third bird had a large granuloma in the oropharynx and a swollen elbow joint. *Yersinia pseudotuberculosis* was isolated from the tissues of all three birds.

10.3.5 Helminths and coccidia

Long thin worms identified as *Diplotrriaena tridens* were present in the anterior airsacs of a blackcap with haemorrhage into the chest and lungs (Images 574-575). The

significance was unclear. Small numbers of unidentified intestinal trematodes (Images 576-579) were detected as incidental findings in three house martins, a swallow and a pied wagtail. Large numbers were also found in a house martin that had died from pseudotuberculosis.

Many coccidial oocysts, presumed to be isosporoid coccidia, were demonstrated in the small intestine of a thin adult pied wagtail with dried faecal material around the vent. Small numbers of intestinal flukes were also present but the coccidial burden was considered to be significant.

10.3.6 Miscellaneous conditions and pathogens

Mycotic pneumonia

A thin waxwing found dead had one normal-looking lung, but the other lung was almost completely replaced by a large cream-coloured mass approximately 3 x 2 cm (Image 572). *Aspergillus fumigatus* was isolated and histopathology revealed a granulomatous pneumonia associated with fungal hyphae.

10.3.7 No diagnosis

No diagnosis was made in 10 ‘other passerines’. Two house martins, two swallows, a chiffchaff, a goldcrest and a waxwing were in moderate or poor condition and had no food in the digestive tract, but the underlying cause was not established. Such birds may have survived earlier undetected trauma or adverse environmental conditions. A goldcrest and swallow in good condition and with food in the digestive may have been the victims of undetected trauma. A treecreeper found dead had an enlarged spleen but no significant bacteria were demonstrated.

10.3.8 Incidental findings

Hippoboscid flies (and occasionally their pupal cases) were found on several house martins (Image 573). Tissues from 41 ‘other passerines’ were screened for *Salmonella* spp., with negative results; liver and intestine from 38 ‘other passerines’ were screened

for *E. albertii* with negative results. Tissues from 19 birds were sent to AHVLA to be screened for avian influenza viruses, but no positive results were reported. Similarly, tissues from 11 birds were sent to AHVLA to be screened for West Nile virus, but no positive results were received.

10.4 Discussion

The great majority of ‘other passerines’ examined had died from trauma or adverse environmental conditions, and the underlying causes were mostly as described in 10.2.1 and 10.2.5 above. One waxwing had died after rupture of the crop, a condition seen more often in pigeons/doves (Chapter 11). This bird also had large numbers of avian gastric yeasts in the proventriculus, and it is possible that the yeasts in the proventriculus had slowed the emptying of the crop, making it more susceptible to trauma. Avian gastric yeasts are discussed more fully in 3.4.3 of this thesis.

Unlike the findings in the species described in Chapters 3-9 and Chapter 11, bacterial or fungal disease was not a major problem in ‘other passerines’. The exception was the diagnosis of pseudotuberculosis, confirmed in three out of eight house martins submitted. The apparent susceptibility to *Y. pseudotuberculosis* of ‘other passerines’ in general and house martins in particular was discussed in 10.2.3 above, and those working in rehabilitation centres should be made aware of this hazard, especially in view of the potential zoonotic significance of *Y. pseudotuberculosis*. It is also interesting to note that the only case of mycotic pneumonia diagnosed in ‘other passerines’ in the current study occurred in a waxwing, and Keymer (1958) made the same diagnosis in a waxwing many years earlier.

In contrast to starlings, blackbirds and thrushes (Chapter 8), corvids (Chapter 9) and pigeons/doves (Chapter 11), internal parasites (helminths and coccidia) were not common problems in ‘other passerines’. The only nematodes noted were the airsac worms *Diplotriaena tridens* in a blackcap that died from intra-thoracic haemorrhage. Although trauma was suspected in this bird, these parasites can cause inflammatory and degenerative lesions in the lungs and other organs (Sterner and Cole, 2008), possibly increasing susceptibility to internal haemorrhage following minor trauma. Intestinal trematodes, possibly *Plagiorchis maculosus* as described by Bock and Janssen (1987), were present in house martins and a swallow, but were not considered to be significant.

Only one bird, a pied wagtail with faecal staining around the vent, had evidence of coccidiosis. With the possible exception of blackcaps, routine preventive treatment for worms and coccidia in ‘other passerines’ in wildlife rehabilitation centres is not justified.

Chapter 11

Diseases of UK pigeons and doves

11.1 Introduction

Pigeons and doves are included in the order Columbiformes. There are over five million breeding pairs of woodpigeons in the UK, making them the commonest member of this order, and nearly one million pairs of collared doves (Musgrove et al., 2013). Rock doves (*Columba livia*) lived on coastal cliffs but were domesticated for food production and latterly for sport, and are now represented by approximately half a million feral pigeons, and the stock dove (*C. oenas*) is a relatively common bird of parkland and farmland, with approximately 260,000 breeding pairs in the UK (Musgrove et al., 2013). In contrast, the UK population of turtle doves is much smaller, with around 14,000 breeding pairs in southern and central England (Musgrove et al., 2013), and in August and September turtle doves leave England for their annual migration back to Africa (Aebischer 2002a). Feral pigeons, woodpigeons and collared doves are in the top five species of wild bird submitted to wildlife rehabilitation centres (Kirkwood and Best, 1998), but the turtle dove is red-listed as being of conservation concern in the UK (Hayhow et al., 2014). The Latin names of the UK birds discussed in this chapter are listed in Appendix I.

11.2 Review of diseases found in UK pigeons and doves

This review includes diseases of wild or feral pigeons and doves, but does not include references to diseases solely described in captive pigeons and doves such as racing pigeons. Further information on diseases of racing pigeons can be found in Pennycott (1996a, 1996b, 2008) and Pees (2008). References that are marked with an asterisk* in the text below contain data that may also be presented in the Results and Discussion sections of this chapter.

11.2.1 Trauma and pasteurellosis

The commonest forms of trauma seen in pigeons and doves presented to wildlife rehabilitation centres were discussed by Stocker (2000). These included predation of collared doves by cats and sparrowhawks, road traffic accidents (especially woodpigeons), shotgun or airgun injuries, and rupture of the crop after a collision. Chitty (2003) also described rupture of the crop in pigeons and doves, commenting that it was uncommon in other groups of birds. An unusual form of trauma to the oesophagus of a woodpigeon was described in which ingested beech nuts appeared to have damaged the mucosa of the oesophagus, resulting in necrosis and secondary bacterial infection (Anon 2013c). Jennings (1961), reviewing the causes of death of 62 columbids, reported that poisoning and infectious disease were more often diagnosed than trauma.

As discussed elsewhere in this thesis, birds that have been attacked by cats and rats and survive the initial trauma may then die from secondary pasteurellosis, caused by *Pasteurella multocida* acquired from the oral cavity of the predators (Macdonald et al., 1981; Stocker 2000).

11.2.2 Poisoning

In his survey into the causes of death of wild birds between 1954 and 1957, Keymer (1958) recorded poisoning by the organochlorine pesticide dieldrin in 59 woodpigeons. This was the commonest cause of death diagnosed in woodpigeons in that survey, and no other species of bird was so badly affected. Jennings (1959) also noted dieldrin poisoning in six woodpigeons, two feral pigeons and a stock dove, and Carnaghan and Blaxland (1957) described “hundreds” of woodpigeons dying from suspected dieldrin poisoning in some locations. Aebischer (2002b) attributed the substantial decline in stock dove numbers in the 1950s to the effects of organochlorine seed dressings, and commented that the population recovered after these products were banned in the 1960s.

Abuse or misuse of pesticides, rodenticides and other products continued to cause mortality in pigeons, especially feral pigeons (WIIS 1998-2004). Fourteen incidents

were recorded between 1998 and 2004, involving alphachloralose; carbamates such as aldicarb, methiocarb, bendiocarb and carbofuran; the anticoagulant rodenticides difenacoum and bromadiolone; and organophosphorus insecticides such as fenthion. Some incidents involved 20-40 feral pigeons.

11.2.3 Avian tuberculosis

Avian tuberculosis is caused by the acid-fast bacillus *Mycobacterium avium*. Early classification combined *M. avium* and the closely related *M. intracellulare* into the *M. avium intracellulare* complex (MAIC) and divided them into different serotypes, but more recently *M. avium* has been divided into different subspecies (Thorel et al., 1990). Avian tuberculosis has long been known to be common in woodpigeons, and McDiarmid (1948) found infection due to acid-fast organisms in 10 out of 66 woodpigeons that had been shot. Miliary foci or caseous nodules were present in a variety of organs including liver, spleen, intestine, mesentery, peritoneum, kidney, pancreas, epicardium, gizzard and pectoral muscle. Eight of the birds were emaciated and had darkening and loss of bloom of the feathers of the mantle (in the centre of the upper back, behind the neck). Soltys and Wise (1967) reported lesions of avian tuberculosis in 28 of 180 shot woodpigeons, and commented that in some birds little normal liver or spleen remained.

Most cases of tuberculosis in birds are caused by strains of *M. avium* that can be cultured using standard techniques (Thoen 1997). However, McDiarmid (1948) and Soltys and Wise (1967) described difficulty in culturing the causal organisms from some affected woodpigeons, and Matthews et al. (1977) subsequently reported that mycobactin, an iron-chelating lipid, was required for the growth of some mycobacteria from woodpigeons. This mycobactin-dependent strain of mycobacterium did not react with *M. avium* or *M. intracellulare* antisera and was sometimes referred to as the “woodpigeon bacillus”. Artificial inoculation of calves with this organism resulted in thickening of the intestinal mucosa and enlargement of the mesenteric lymph nodes, similar to lesions

seen in cattle with Johne's disease, another mycobacterial disease (Matthews and McDiarmid, 1979; Collins et al., 1985).

Using a large battery of different growth requirement tests and enzymatic activities, Thorel et al. (1990) divided *M. avium* into three subspecies; *M. a. avium*, the commonest cause of avian tuberculosis in birds and animals; *M. a. paratuberculosis*, the cause of Johne's disease in cattle and sheep; and *M. a. silvaticum*, the mycobactin-dependent cause of some cases of avian tuberculosis in woodpigeons. Molecular techniques looking at the presence or absence of different nucleotide sequences in the organisms' genomes reinforced these differences, with insertion sequence IS900 found in *M. a. paratuberculosis*, IS901 found in *M. a. avium*, and IS902 found in *M. a. silvaticum* (Kunze et al., 1992; Moss et al., 1992).

11.2.4 Avian pox

A comprehensive review of avian pox in woodpigeons in the 1950s and 1960s was provided by Blackmore and Keymer (1969). They noted that pox was diagnosed in 17 out of 284 woodpigeons examined between 1954 and 1962, and commented that other birds were visibly affected but not submitted. Disease was seen in adult and immature birds, especially in the winter months. Vesicles, pustules or granulomatous nodules were typically seen around the eyelids, on the beak, and on legs and feet. Lesions were also found on the wings and at the vent. Murton (1965) described several outbreaks of avian pox in woodpigeons, and illustrated lesions at the head and carpal joint.

11.2.5 Trichomonosis

In the early 1900s, there were reports of a disease causing the death of thousands of woodpigeons in England in autumn and winter (Murton 1965). Thick layers of yellow debris were described at the base of the tongue, the pharynx and the oesophagus, sometimes almost obstructing the upper digestive tract. Initially the condition was described as "woodpigeon diphtheria" and avian pox was suspected, but the most likely cause was infection with the protozoan parasite *Trichomonas gallinae* (Murton 1965).

The importance of trichomonosis in woodpigeons was reported by McDiarmid (1956) and Keymer et al. (1962), and in collared doves by de Gruchy (1983), and there were reports of widespread losses from trichomonosis in 1996 (Mead 1996) and in 2002 (Duff 2002). Cousquer (2003) analysed the records of a wildlife hospital over a five-year period and noted that 25 of 665 collared doves had lesions of oral trichomonosis, as did 79 of 1026 woodpigeons. Both species showed seasonal peaks in the autumn months. Trichomonosis is also common in racing pigeons (Pees 2008), where the condition is referred to as “canker”, and was diagnosed in feral pigeons after approximately 60 had been found dead (Anon 2013d).

Almost one hundred years after the reports of presumed trichomonosis in woodpigeons in England, there was increased interest in trichomonosis following the appearance of the disease in finches (see Chapters 6 and 7 of this thesis for a full discussion). Using molecular techniques such as PCR amplification and ribosomal RNA sequencing, Lennon et al. (2013) detected *T. gallinae* in 86% of collared doves, 86% of turtle doves, 47% of woodpigeons and 40% of stock doves from farmland sites in England, showing how widespread the organism was in columbids. PCR products from eleven woodpigeons, nine turtle doves and one stock dove were sequenced at the ITS1/5.8S/ITS2 ribosomal region (internal transcribed spacer regions 1 and 2), and three different strains were identified, one of which (from four turtle doves and a woodpigeon) was identical to the strain causing disease in UK finches. With the exception of one bird, a woodpigeon with a large caseous mass in the oropharynx, the birds appeared healthy.

Similar findings were made by Chi et al. (2013), who obtained ITS region sequences of *T. gallinae* from 100% of feral pigeons and stock doves tested, 89% of collared doves and 71% of woodpigeons. All the birds were from England, with or without gross lesions of trichomonosis. Sequencing at the ITS region showed that about half of the isolates from feral pigeons, woodpigeons and collared doves were identical to the UK finch strain. These studies supported the hypothesis that the recent trichomonosis

epidemic in finches resulted from a spillover of infection from columbids, possibly at garden feeders (Robinson* et al., 2010; Lawson* et al., 2012b).

Stockdale et al. (2015) found lesions typical of trichomonosis in the oropharynx of one adult and two nestling turtle doves, and demonstrated the same clade of *T. gallinae* as that responsible for the finch epidemic. Although there is no suggestion that trichomonosis is currently endangering the populations of pigeons and doves in the UK, the condition has been shown to limit the population size of the endangered Mauritius pink pigeon (*Columba mayeri*) on the Indian Ocean island of Mauritius (Bunbury et al., 2008). The report by Stockdale et al. (2015) of fatal trichomonosis in turtle doves in England is, therefore, of some concern.

11.2.6 Pigeon paramyxovirus-1 (PPMV-1) infection and avian influenza

Newcastle disease of poultry is caused by avian paramyxovirus-1 (APMV-1), and occasionally this virus has spilled over into captive pigeons (Stewart 1971). However in the 1980s, a variant strain of APMV-1, referred to as pigeon paramyxovirus-1 (PPMV-1) spread rapidly throughout lofts of racing and show pigeons in Europe, causing green watery diarrhoea and central nervous system (CNS) signs such as incoordination, torticollis and paresis of the legs and wings (Aldous et al., 2004). Lofts of racing pigeons in the UK were affected from 1983, and the following year there were multiple outbreaks in poultry in the UK (Alexander et al., 1985). The source of infection was shown to be feral pigeons infected with PPMV-1, living in large feed stores and contaminating the feed ingredients with their faeces (Alexander et al., 1984). Lister et al. (1986) provided further details of the role played by feral pigeons in spreading disease to poultry, and described green diarrhoea and a variety of CNS signs in affected feral pigeons. PPMV-1 has also been isolated from a mass mortality incident affecting feral pigeons and woodpigeons in England (Anon 2011d), and from two incidents in which several collared doves were found dead in gardens in England (Anon 2011b). Bonfante et al. (2012) showed that different lineages of PPMV-1 were circulating in collared doves in Italy, sometimes associated with high mortality.

Pigeons and doves are relatively resistant to the effects of avian influenza viruses (Klopfleisch et al., 2006). However, some feral pigeons experimentally challenged with high numbers of highly pathogenic avian influenza (HPAI) H5N1 virus died after showing anorexia, depression and neurological signs (Klopfleisch et al., 2006). In other pigeons, no clinical signs were observed and viral shedding was of short duration and intensity (Brown et al., 2009). No records of naturally-acquired avian influenza infection in birds of the order Columbiformes in the UK were found when preparing this thesis.

11.2.7 Infection with *Salmonella* spp., *Campylobacter* spp. and *Yersinia pseudotuberculosis*

Feral pigeons have long been considered a possible source of salmonellae for humans, and Farrant et al. (1964), cited by Wilson and Macdonald (1967), recovered *S. Typhimurium* from 43 of 246 feral pigeons in London. Pennycott* et al. (2006) diagnosed salmonellosis as the cause of death of four feral pigeons examined between 1995 and 2003; affected birds had pathological lesions such as pericarditis, perihepatitis, pneumonia and arthritis, and *S. Typhimurium* DT2 or DT99 was isolated on direct culture. These authors did not isolate salmonellae from 19 woodpigeons and 15 collared doves from Scotland, and Lawson et al. (2010) did not recover the organism from three woodpigeons and 16 collared doves from England/Wales. The significance of *S. Typhimurium* DT56 isolated from a woodpigeon and collared dove from northern England was unclear (Hughes et al., 2008). Wilson and Macdonald (1967) described concurrent salmonellosis and pox in a woodpigeon, and reported the recovery of *S. Gallinarum* (the cause of fowl typhoid of poultry) from two woodpigeons.

Food-poisoning caused by *Campylobacter jejuni*, and to a lesser extent *C. coli*, is now more common than salmonellosis in humans, and pigeons have been shown to carry campylobacters. Fenlon (1983) isolated *C. jejuni* from up to 40% of feral pigeons in the UK; Megraud (1987) recovered campylobacters, mostly *C. jejuni*, from 53% of feral pigeons sampled in France; and Vazquez et al. (2010) demonstrated *C. jejuni* in 69% of

feral pigeons in Spain. Other studies have shown lower levels of carriage, for example Hughes et al. (2009) detected *C. jejuni* in only two out of 47 feral pigeons tested from the north of England (4%). Bianchini et al. (2014a; 2014b) reported the recovery of *C. jejuni* from 22% of feral pigeons from dairy farms in Italy, but using multi-locus sequence typing (MLST) showed that the pigeon isolates formed a pigeon-specific clone, with no evidence of spread to the cattle or the milk. Overall, no evidence was found to indicate that pigeons and doves are significant reservoirs for *C. jejuni* in humans, and Sheppard et al. (2009) concluded that most cases of *Campylobacter* food-poisoning in humans were acquired from poultry or ruminants.

In his review of yersiniosis (pseudotuberculosis) in wildlife, Mair (1973) recorded that the woodpigeon was the species of UK wild bird from which *Yersinia pseudotuberculosis* was most frequently isolated between 1961 and 1971. He commented that infected birds excreted large numbers of organisms in their faeces, contaminating the crops on which they fed and posing a threat to laboratory animals, farm livestock and zoo animals and birds fed on greenfoods. Other species of pigeons and doves are also susceptible, and Clapham (1953) described an outbreak of yersiniosis in stock doves (and hares) in the south of England, in which small caseous nodules were present in the liver and spleen of most of the 42 stock doves examined. The birds were found dead under their roosts and were in good body condition. Deaths occurred in December and January, reducing in February.

11.2.8 Infection with *Chlamydia (Chlamydophila) psittaci*

Chlamydiosis in collared doves in the winter of 1981/1982 was described by Gough and Bevan (1983). Affected birds were in poor body condition and had severe keratoconjunctivitis of one or both eyes. Enlargement of the liver and spleen was noted at necropsy, and some birds had necrotic foci in the liver. *C. psittaci* was also isolated from pooled intestines from woodpigeons feeding near the collared doves. Further cases were reported by de Gruchy (1983), who found lesions of concurrent trichomonosis in some birds. The zoonotic implications for those handling sick and dead birds were

stressed. The importance of chlamydiosis in birds of the order Columbiformes was noted by Sharples and Baines (2009), who tested wild birds in a wildlife rehabilitation centre in England. Five of 43 birds tested by PCR for *C. psittaci* were positive, and all were columbiforms; three feral pigeons, one collared dove and one woodpigeon.

Chlamydiosis in collared doves, feral pigeons and a woodpigeon was described by Pennycott* et al. (2009), and Beckmann et al. (2014a) gave a detailed description of chlamydiosis in two collared doves, in which the main postmortem features were combinations of hepatomegaly, splenomegaly, pericarditis and airsacculitis. The latter authors also genotyped isolates of *C. psittaci* from 27 wild birds, including the two collared doves described above, and found that although most isolates were Genotype A, the collared dove isolates were Genotype E. In a review of chlamydial infections of feral pigeons in Europe, Magnino et al. (2009) reported that some studies found up to 33% of feral pigeons were PCR-positive for *C. psittaci*, and that most isolates genotyped were Genotype B or Genotype E. While most were latent carriers and appeared healthy, stress factors such as concurrent disease, overcrowding and malnutrition could increase shedding and precipitate clinical disease, resulting in oculo-nasal discharges and diarrhoea (Magnino et al., 2009). Higher levels of infection of feral pigeons with *C. psittaci* were reported by Vazquez et al. (2010), who detected the organism in 53% of feral pigeons in Madrid, Spain. As in other papers, the zoonotic risks to humans from feral pigeons were highlighted.

11.2.9 Internal parasites – helminths and coccidia

Keymer et al. (1962) reported the presence of hairworms in the intestine of woodpigeons and described them as *Capillaria longicollis*, *C. columbae* and *C. retusa* (now considered a synonym for *C. anatis*). Using the classification adopted by Anderson (1992), the hairworms found in the intestine of pigeons are likely to be *Aonchotheca caudinflata* (previously *C. longicollis*) or *Baruscapillaria obsignata* (previously *C. columbae*). The former has an indirect life cycle involving an earthworm and the latter has a direct life cycle. Earthworms do not form a major part of the diet of woodpigeons

(Murton 1965), suggesting that these hairworms would be *B. obsignata*. The tapeworms *Hymenolepis columbae*, *Raillietina tetragona* and *R. columbae* were identified in woodpigeons by Keymer et al. (1962), and these authors considered *H. columbae* to be the cause of death of two of the birds. Tapeworms of the genera *Hymenolepis* and *Raillietina* that are found in poultry employ different species of flies, beetles and ants as intermediate hosts (Trees 2008), and the same may be true for columbids. Coccidia of the species *Eimeria labbeana* and *E. columbarum* are commonly found in racing pigeons (Pennycott 1996a) and Keymer et al. (1962) noted that these species were also present in woodpigeons as incidental findings.

11.2.10 External parasites

Blood sucking mites, feather mites, quill mites, ticks, lice, fleas and hippoboscids louse flies can all be found in the nests of pigeons and doves, or on the birds themselves (Murton 1965). Among the mites and ticks listed by Murton were the red mite (*Dermanyssus gallinae*), feather mites of the family Analgesidae, the quill mite *Syringophilus* sp. and the sheep tick *Ixodes ricinus*. Another tick found on pigeons and doves was *Ixodes frontalis* (Monks et al., 2006). The commonest lice described by Murton belonged to the genera *Columbicola*, *Campanulotes* and *Goniocotes*, and *Ceratophyllus gallinae* was the commonest flea. The hippoboscids louse fly *Ornithomyia avicularia* is frequently found on woodpigeons or in their nest, and is unusual in that it will often crawl through the feathers rather than fly off if disturbed. If it does fly off, it seldom goes far and may then crawl over the face or arms of nearby humans (Murton 1965). The pathological significance of the external parasites listed above is often unclear, but large numbers of blood-sucking parasites such as red mites, ticks, fleas and hippoboscids louse flies may have an adverse effect and could be involved in the transmission of some diseases. *Ixodes frontalis* can also be associated with a “tick-related syndrome” – see below.

11.2.11 Miscellaneous conditions and pathogens

Adverse environmental conditions

During the cold winter of 1962/1963, Ash and Sharpe (1964) carried out postmortem examinations on over 200 wild birds. Woodpigeons were among the six species of bird most often found dead, and no underlying infectious disease was detected in them.

Mycotic pneumonia / airsacculitis / hepatitis

Mycotic pneumonia in wild birds was discussed by McDiarmid (1955). Of 18 cases confirmed in a ten-year period, 14 occurred in woodpigeons. *Aspergillus fumigatus* was isolated from 13 woodpigeons and *A. nidulans* from one bird. McDiarmid commented that respiratory tract lesions in woodpigeons were not as obvious as in some other species, and necrotic nodules that could be confused with those of avian tuberculosis were present in the liver of some woodpigeons.

Metabolic bone disease

Deformities of the beak, sternum and tibiotarsus were frequently seen in young collared doves submitted to wildlife rehabilitation centres, especially in January to March (Cousquer et al., 2007). Affected birds were often unable to stand or fly because the poorly mineralised bones bent easily, and some affected birds also had retained keratin sheaths and stress marks on the feathers of the wings and tail. Rickets caused by vitamin D deficiency was diagnosed on the basis of bone histopathology. Cousquer et al. (2007) suggested that the rearing of young birds during the winter months could result in dietary deficiencies or impaired absorption of vitamin D, because of reduced exposure to ultraviolet light during the dark winter months. The condition was also described in immature woodpigeons that died in a rehabilitation centre in September, in which the legs were soft, bendy and deformed, with pathological fractures (Anon 2010a). An inappropriate diet deficient in calcium and possibly vitamin D was thought to be the cause.

Nutritional muscular dystrophy

Degeneration of the pectoral muscles of a collared dove was noted by Baker (1977). The bird was unable to fly and trailed its wings along the ground, and the histopathological appearance of the muscle was reported to be similar to that of nutritional muscular dystrophy (white muscle disease) of mammals, resulting from deficiencies of selenium and/or vitamin E.

Tick-related syndrome

A condition of UK birds associated with the tick *I. frontalis* was described by Monks et al. (2006). Affected birds were typically found dead or severely depressed, with haemorrhage and oedema of the head and neck associated with the presence of ticks. The birds most frequently affected were columbiforms and raptors (Monks et al., 2006), and Chitty (2003) illustrated a typical case in a collared dove. The pathogenesis was unclear.

Mycoplasma spp. in pigeons and doves

The isolation of *Mycoplasma columbinum* and *M. columborale* from feral pigeons in the UK was reported by Jordan et al. (1981). The birds appeared healthy, but both species of mycoplasma were isolated from seven out of 10 birds tested, mostly from the oropharynx and oesophagus. These authors speculated that a third species, *M. columbinasale*, may also have been present but overgrown by the more-rapidly growing *M. columbinum* and *M. columborale*. Conjunctival and oropharyngeal swabs from two woodpigeons from Scotland were among those screened for mycoplasmas by culture and by PCR for *M. gallisepticum* and *M. synoviae* (Pennycott* et al., 2005b); *M. gallisepticum* and *M. synoviae* were not detected in either bird, and the isolation of *M. columbinum* from the oropharynx of one bird was not considered to be significant.

Haemoparasites

Murton (1965) noted that blood parasites of the genus *Haemoproteus* were common in woodpigeons in the UK, and that *Leucocytozoon marchouxi* was also found in some

woodpigeons. He commented that these parasites seldom caused problems in the UK except under exceptional circumstances, but Earle et al. (1993) reported deaths caused by *H. columbae* in bleeding heart doves (*Gallicolumba luzonica*) in South Africa, and Peirce et al. (1997) described mortality associated with *L. marchouxi* in the Mauritius pink pigeon. *Haemoproteus* spp. in pigeons and doves are usually spread by hippoboscids louse flies (Atkinson 2008), and *L. marchouxi* by black flies of the family Simuliidae (Forrester and Greiner, 2008).

Reproductive tract disorders

Egg yolk peritonitis was diagnosed in a collared dove by Keymer (1958), who noted that this was of special interest because the affected bird was one of the first to breed in the UK.

Botulism

Botulism resulted in heavy mortality in birds at St. James's Park, London, in the summer of 1969 (Keymer et al., 1972). Although mostly ducks were affected, these authors also noted deaths in other birds including woodpigeons but gave no further details.

11.3 Diseases of pigeons and doves examined at Ayr DSC 1994 to 2013 – results

11.3.1 Bird species, numbers and locations

Two hundred and twelve pigeons and doves were examined between January 1994 and December 2013. Of those for which a location was given, 95% were found in the south of Scotland and 5% in the north. A summary of the species examined and the conditions diagnosed can be found in Tables 11.1 and 11.2. The Materials and Methods are described in Chapter 2 and the diagnostic criteria used are listed in Appendix II.

Table 11.1: Pigeons and doves examined.

Species	Number examined
Feral pigeon	109
Woodpigeon	62
Collared dove	40
Stock dove	1
Total	212

Table 11.2: Conditions* diagnosed in pigeons and doves (n=212).

Condition	Number of birds	% (95% confidence intervals)
Trichomonosis (presumed or confirmed)	39	18.4 (13.6-24.4)
Trauma	31	14.6 (10.3-20.3)
Pigeon paramyxovirus-1 (PPMV-1) infection	18	8.5 (5.2-13.3)
Metabolic bone disease	14	6.6 (3.8-11.1)
Avian tuberculosis	12	5.7 (3.1-9.9)
Chlamydiosis/chlamydiasis	10	4.7 (2.4-8.8)
Arthritis, cellulitis and granulomata	10	4.7 (2.4-8.8)
Pox (presumed or confirmed)	9	4.2 (2.1-8.2)
Significant helminthosis	6	2.8 (1.2-6.3)
Salmonellosis	5	2.4 (0.9-5.7)
<i>Spirochete</i> (<i>Hexamita</i>) sp. infection	5	2.4 (0.9-5.7)
Reproductive tract disorder	5	2.4 (0.9-5.7)
Miscellaneous conditions and pathogens:		All <2%
Inclusion body hepatitis	4	
Adverse environmental conditions	4	
Mycotic pneumonia / airsacculitis	4	
Circovirus infection	3	
<i>Pasteurella multocida</i> infection	3	
<i>E. coli</i> septicaemia	2	
Skeletal disorder not otherwise specified	2	
Necrotic enteritis	1	
Digestive tract condition not otherwise specified	1	
Neoplasia	1	
Respiratory tract condition not otherwise specified	1	
No diagnosis	42	19.8 (14.8-25.9)
Incidental findings:		Not applicable
<i>Salmonella</i> spp. through selenite only	2	
Small to large numbers of coccidial oocysts	Not recorded	
Helminth burdens not considered significant	Not recorded	
<i>Haemoproteus columbae</i> and <i>Leucocytozoon marchouxi</i>	1	
Tissues screened for avian influenza virus – no positive results	56	
Tissues screened for West Nile virus – no positive results	72	

*Not all birds were examined for all conditions, and some birds had multiple conditions

11.3.2 Trauma

Trauma was responsible for the death or culling of 12 feral pigeons, 14 woodpigeons and five collared doves. Injuries to the head, neck, chest, back and wings were commonly seen, and in some birds there was rupture of abdominal organs or internal haemorrhage. Possible causes included road traffic accidents, window collisions, firearm wounds and predator attacks. In most cases no predisposing factors were identified, but one collared dove had pre-existing trichomonosis and one thin woodpigeon had fluid intestinal contents associated with *Spironucleus* sp. Some birds exhibiting CNS signs may have had undetected lesions of head trauma, but such cases were recorded as “no diagnosis”.

11.3.3 Avian tuberculosis

Avian tuberculosis was diagnosed in ten adult woodpigeons, one collared dove and one feral pigeon. All the woodpigeons and the collared dove were submitted in the months December to May, and seven occurred in April or May. In woodpigeons, small to large nodules of a cream, yellow or orange colour were most frequently found in the liver and intestine ([Images 580, 583, 585, 586, 590, 596, 597, 598](#)), and in three woodpigeons one lung was almost completely replaced by a large pale flat mass ([Images 581, 587, 598](#)). The spleen was sometimes greatly enlarged and pale ([Images 582, 588, 589, 598, 603](#)), with nodules visible on cut surface. Nodules in the myocardium and/or pericarditis were seen in two birds ([Images 597, 600-602](#)), in another bird a large caseous mass was found beneath the skin in front of one eye ([Images 591-593](#)), and a single large granular nodule on a thoracic airsac was the only evidence of avian tuberculosis in one bird. The mucosa of the duodenum of four woodpigeons was orange-yellow, thickened, fissured and necrotic ([Images 594, 599](#)), and in one bird the mucosa of the crop and oesophagus was also thickened and yellow ([Image 595](#)). In one woodpigeon and a collared dove the only abnormality found was a large yellow to fawn multi-nodular mass within the tissues overlying one carpal joint ([Images 604-608](#)).

Extensive lesions of visceral and articular gout were present in one woodpigeon with avian tuberculosis (Images 610-613); both legs were considerably swollen, with white subcutaneous urates clearly visible through the skin. Urates were also present in the tubules of the kidneys; in the hip, hock, knee and shoulder joints; and in the tissues overlying the leg and breast muscles and the cervical spine. The feral pigeon with avian tuberculosis had a large caseous mass between the skin and the crop, and a small fistula extended from the crop into the mass (Images 609-610). A diagnosis of avian tuberculosis was made in all twelve birds, based on the presence of large numbers of acid-fast bacilli on microscopic examination of Ziehl-Neelsen stained smears from affected tissues (Images 614-615).

11.3.4 Avian pox

Lesions typical of avian pox were found in nine woodpigeons. Adult and immature birds were affected, and no seasonal distribution was apparent. Pox was confirmed in seven of the birds by demonstration of poxvirus by electron microscopy, by histopathology (Image 629) or by PCR, but no confirmatory testing was carried out in the remaining two birds. Single or multiple nodules were found around the eyes, on the mandibles, on the body wall or on the digits of eight birds (Images 616-625). One bird with a single nodule on the head also had a large dry mass with yellow interior on the carpus and wing tip (Images 626-627), and another bird had similar masses overlying one elbow joint and wing tip. Two birds had enlarged livers; *S. Typhimurium* DT161 was isolated from the liver of one bird in which histopathology demonstrated a bacterial hepatitis, and in the other bird a high proportion of the leucocytes in the liver sinusoids were parasitised by *Leucocytozoon marchouxi* gametocytes (Images 630-632). Lesions of avian pox were not detected in any of the feral pigeons or collared doves examined.

11.3.5 Trichomonosis

The commonest diagnosis made in collared doves was presumed or confirmed trichomonosis (Images 638-639), diagnosed in 21 out of 40 (52.5% [95% CI: 36.3-68.2]) collared doves submitted. Yellow caseous lesions were found in the oropharynx and

upper oesophagus, frequently extensive and resulting in partial occlusion of the upper digestive tract. In one bird the lesions were in the lower oesophagus between the crop and the proventriculus. Cases were seen throughout the year, with the greatest number in the fourth quarter of the year. Trichomonosis was also a common finding in woodpigeons (Images 633-637), seen in 12 out of 62 (19.3% [95% CI: 10.8-31.7]) woodpigeons examined. Lesions were similar to those observed in collared doves, and again the greatest numbers of cases were in the fourth quarter of the year. The condition was much less common in feral pigeons (4.6% [95% CI: 1.7-10.9] of submitted birds) but the one stock dove examined had extensive lesions of trichomonosis (Images 640-642). One side of the face of this adult bird was swollen and crusted, obscuring one eye; there was much yellow or cream debris in the oropharynx, nasal passages and in one infra-orbital sinus, hollow in places and extending to the subcutaneous tissues over the mandible on that side. Trichomonosis was confirmed in this bird by PCR.

11.3.6 Pigeon paramyxovirus-1 (PPMV-1) infection

Signs of damage to the central nervous system (CNS) such as ataxia, incoordination, torticollis, head tremors and circling (Images 643-644) were commonly reported in wild columbids that had died or been culled and submitted for postmortem examination. In eighteen feral pigeons, PPMV-1 infection was confirmed by serology or virus isolation, but the disease was suspected in another 28 feral pigeons in which no further tests were carried out or in which serology or virology results were inconclusive or negative – these cases were coded as “no diagnosis”. Confirmed cases were often thin and had been passing green fluid faeces, and some carried significant burdens of hairworms, tapeworms or coccidia. One bird with confirmed PPMV-1 infection had a concurrent mycotic pneumonia from which *Aspergillus fumigatus* was isolated.

11.3.7 Inclusion body hepatitis and circovirus infection

Inclusion body hepatitis (IBH), most likely caused by an adenovirus infection, was confirmed on histopathological examination of the liver of four feral pigeons. One bird had been observed to be ill in a garden on one day and found dead the next; this bird had

an enlarged liver, severe perihepatitis and pericarditis ([Image 648](#)). Another bird found dead had an enlarged liver with haemorrhagic speckling and very fluid intestinal contents, and a further two thin feral pigeons found alive but moribund also had very watery intestinal contents. Two birds with IBH were PCR-positive for *Chlamydia psittaci*. Histopathological examination of the livers of the four birds demonstrated widespread necrosis of hepatocytes associated with many basophilic intranuclear inclusion bodies, consistent with an adenovirus infection ([Images 649a, 649b](#)).

Botryoid inclusions, severe lymphoid necrosis and granulocytic infiltration were detected in the bursa of Fabricius of three immature feral pigeons. These lesions were typical of circovirus infection (Pennycott 2008). Two birds had caseous cores in the bursa ([Image 650](#)), and in the third bird the bursa was much reduced in size.

11.3.8 Infection with *Salmonella* Typhimurium and *Escherichia albertii*

Salmonellosis was diagnosed in four feral pigeons during the study, between the months of August and December. *S. Typhimurium* DT2 was recovered from the elbow joint of a bird with a purulent arthritis, and the same phage type was recovered from the viscera of a bird with multiple nodules in the liver ([Images 645-646](#)) and lungs. *S. Typhimurium* DT99 was isolated from a bird with an abdominal abscess, and from a bird showing torticollis and which had pneumonia and pleurisy ([Image 647](#)), perihepatitis and multiple foci of necrosis in the liver. Concurrent salmonellosis and pox was diagnosed in a woodpigeon with lesions of pox on the head, legs and feet and that had an enlarged liver at necropsy. *S. Typhimurium* DT161 was isolated in heavy growth from the liver, and histopathology demonstrated a bacterial hepatitis in addition to confirming pox lesions on the head. The recoveries through enrichment media of *S. Liverpool* from a feral pigeon that died from trauma and of *S. Typhimurium* DT99 from a feral pigeon with suspected PPMV-1 infection were considered to be incidental findings. Including the cases described above, *Salmonella* spp. were isolated from 6 out of 88 feral pigeons (6.8% [95% CI: 2.8-14.8]), 1 out of 48 woodpigeons (2.1% [95% CI: 0.1-12.5]), but not from 25 collared doves or one stock dove.

Liver and intestine from 144 pigeons and doves were screened for *E. albertii*. Potentially-pathogenic *E. albertii* with the API-20E profile of 4144102 or 5144102 were isolated from three feral pigeons (Appendix VI). Serology for O86:K61 was carried out on two of the isolates with negative results, and they were considered to be non-pathogenic. *E. albertii* is discussed more fully in Chapter 5.

11.3.9 Chlamydiosis

Chlamydiosis contributed to the deaths of five feral pigeons, all submitted between May and September. In four of the birds, the intestines were distended with green fluid or mucoid contents, and four birds had combinations of hepatomegaly, epicarditis, pericarditis, perihepatitis or airsacculitis. Two birds had concurrent IBH, one had large numbers of coccidial oocysts in the intestine, one had metabolic bone disease, and *Listeria monocytogenes* was isolated from the liver of one bird. Combinations of fluid intestinal contents and/or purulent airsacculitis were present in four collared doves that died from chlamydiosis. Unlike the feral pigeons, cases were spread throughout the year, with submissions in January, June and November. Two of the birds were adults, and two were immature and also had soft bones caused by metabolic bone disease. *C. psittaci* may also have contributed to the death of a thin immature woodpigeon with large numbers of tapeworms distending the duodenum – the liver of the bird was dark and enlarged, and histopathology showed multiple foci of liver necrosis and heavy infiltration by heterophils. Chlamydiosis was diagnosed in the above 10 birds by demonstration of *C. psittaci* by PCR. Samples from a further five feral pigeons, five woodpigeons and three collared doves were screened for *C. psittaci* by PCR with negative results.

11.3.10 Helminths and coccidia

Small to large numbers of eggs or adult worms of the subfamily Capillariinae (hairworms) were detected in the intestinal contents of many feral pigeons and some woodpigeons ([Images 656-662](#)), but only in one collared dove. In most cases the

hairworms were incidental findings, but in two feral pigeons and two woodpigeons the burdens were considered to be significant; these birds were all thin, one feral pigeon had concurrent spironucleosis, one woodpigeon also had pox, and in one woodpigeon large numbers of hairworms were associated with necrosis of the mucosa of the duodenum.

Unidentified tapeworms were detected in 12 woodpigeons and four feral pigeons, including one immature woodpigeon in which the duodenum was distended by many tapeworms and watery yellow fluid; this bird was also PCR-positive for *C. psittaci*, but the large numbers of tapeworms were thought to have contributed to the watery intestinal contents.

One woodpigeon with trichomonosis had a haemorrhagic enteritis associated with the trichostrongyle nematode *Ornithostrongylus quadriradiatus*. The contents of the distal small intestine were haemorrhagic, and smears demonstrated large numbers of thin worms approximately 1 cm in length. Moderate numbers of this parasite were also found in a stock dove with trichomonosis. The worms were identified as *O. quadriradiatus* on the appearance of the bursal rays and spicules of the male worms, and the presence of an inflated cuticle at the anterior end of the worms (Rose and Keymer, 1958) (Images 663-668). Small numbers of unidentified intestinal trematodes were detected in a feral pigeon and a woodpigeon.

Coccidial oocysts (Images 651-655) were frequently found in the intestinal contents of feral pigeons and woodpigeons, and occasionally in collared doves, but were not considered significant. Further details of the helminths and oocysts described above can be found in Appendix III.

11.3.11 *Spironucleus (Hexamita)* sp.

Large numbers of motile protozoa with the morphology of *Spironucleus (Hexamita)* sp. were demonstrated in intestinal smears from four thin feral pigeons submitted between August and November. The small intestine of all the birds was distended with fluid

contents, and in one bird the intestinal wall was thickened; this bird also had large numbers of intestinal hairworms. Moderate numbers of presumed *Spironucleus* sp. were detected in a thin adult woodpigeon that had been euthanased because of extensive limb fractures.

11.3.12 Metabolic bone disease and other skeletal defects

Ten immature collared doves had severe leg deformities when found dead or when euthanased at a wildlife rehabilitation centre. Birds typically had marked bilateral bowing of the tibiotarsus bones and some had pathological fractures of the proximal tibiotarsus with extensive callus formation. Two birds had concurrent chlamydiosis. Histopathological examination of the tibiotarsus of two affected birds demonstrated thickening of the growth plate due to a widened zone of proliferative cartilage, suggestive of hypocalcaemic rickets. In a third bird there was reduced mineralisation of the zone of hypertrophy and fibrosis of the metaphysis, but no conclusions could be drawn as to the cause. Two young woodpigeons and two young feral pigeons, one with concurrent chlamydiosis and IBH, were similarly affected. Seven cases occurred in March to May and six in November to December, with the remaining case (a feral pigeon with concurrent chlamydiosis and IBH) submitted in September.

A young feral pigeon was culled because of marked scoliosis of the cervical spine, and another was culled because the distal femur and proximal tibiotarsus were rotated through approximately 180°. As in the cases of metabolic bone disease described above, one was submitted in April and one in December.

11.3.13 Arthritis, cellulitis and granulomata

Seven birds (five woodpigeons, one feral pigeon and one collared dove) had swollen joints and peri-articular tissues containing purulent or caseous material (Images 669-670). The elbow joints were most commonly affected, but involvement of the carpal, hock and stifle joints was also seen. Histopathology on tissues from two birds confirmed a bacterial arthritis, synovitis and osteomyelitis, but bacteriology on the infected tissues

from these seven birds did not demonstrate any significant organisms, other than *E. coli* of questionable significance from some birds. *E. coli* was also recovered from the subcutaneous tissues of a collared dove that survived a cat attack but developed green cellulitis of the head and neck; in addition, the bird had significant trichomonosis of the oropharynx.

Two feral pigeons each had a large granuloma approximately 4-5 cm in diameter in the pectoral muscles. In one bird the mass was fawn-coloured and extended into the crop and through the overlying skin (Images 671-672). Cross-section of the mass from the second bird revealed multiple alternating layers of red and white tissues. Histopathology on tissues from both birds demonstrated a bacterial granuloma, with progressive enlargement and tissue necrosis in the bird with multiple layers. Mixed bacteria of no significance were isolated, and in both birds the granulomata may have been secondary to initial trauma to the breast muscle.

11.3.14 Miscellaneous conditions and pathogens

Adverse environmental conditions

Two woodpigeons were submitted in January, representative of approximately 70 woodpigeons found dead over a few days when there was prolonged snow and ice and temperatures fell to minus 10°C. No food was present in the digestive tracts, and the lungs were congested and oedematous. Two nestling woodpigeons submitted in August were thought to have fallen or been blown out of their nest.

Mycotic pneumonia / airsacculitis

Mycotic nodules in the lungs or airsacs were found in four feral pigeons, two of which also had suspected or confirmed concurrent PPMV-1 infection. *Aspergillus fumigatus* was isolated from three of the birds, but no fungi were cultured from one bird; histopathology of the lung of this bird showed a mycotic pneumonia associated with irregular hyphae suggestive of a zygomycetic fungus such as *Mucor* sp. This bird died seven days after admission to a wildlife rehabilitation centre, and it was unclear if the

mycotic pneumonia was present in the bird prior to admission or had been acquired following admission.

Respiratory tract conditions not otherwise specified

A thin adult feral pigeon found dead had areas of consolidation in both lungs. No significant bacteria were isolated but histopathology demonstrated an acute fibrino-granulocytic pneumonia associated with large rod-shaped bacteria, sometimes filamentous and branching.

Pasteurella multocida and Yersinia pseudotuberculosis infection

In contrast to the situation with garden birds (Chapter 3), *P. multocida* was not isolated from any pigeons or doves that died from trauma. The organism was, however, recovered from the brain and liver of three feral pigeons exhibiting CNS signs, one of which also had necrotic foci in the liver. Unfortunately, brain histopathology was not carried out in these cases, and the association between the CNS signs and *P. multocida* was not established. *Yersinia pseudotuberculosis* was not isolated from any of the pigeons and doves examined in this study.

Reproductive tract disorders

Five feral pigeons died from impaction of the oviduct; in one bird a substantial ventral hernia was also present. Two cases occurred in May, and three in September or October.

Necrotic enteritis

Necrotic enteritis was diagnosed in an adult feral pigeon that died shortly after admission to a wildlife rehabilitation centre. The small intestine was ulcerated, and yellow necrotic foci were visible on the serosal and mucosal surfaces. Gram-stained smears from the intestine revealed many Gram-positive bacilli, and *Clostridium perfringens* was isolated on anaerobic culture. No internal parasites were demonstrated and the factor or factors precipitating the necrotic enteritis were not determined.

Digestive tract conditions not otherwise specified

An adult male feral pigeon was found with a prolapsed cloaca surrounded by dried urates. The bird was thin and had a large caseous mass in the distal small intestine and rectum, but no significant organisms were demonstrated on cultures or stained smears.

E. coli septicaemia

A diagnosis of secondary *E. coli* septicaemia, primary cause unknown, was made in two immature feral pigeons. One bird had a stained vent, loose faeces and a purulent airsacculitis and peritonitis, and the other bird had marked enlargement of the liver and spleen. Heavy growths of *E. coli* were isolated from the heart, liver and intestine of both birds, but tests for a primary chlamydial or viral infection were not carried out because of financial constraints.

Neoplasia

An adult feral pigeon had a pedunculated mass on the outer surface of one wing near the carpus. The mass measured 5.0 x 3.5 x 3.5 cm, was fawn and pink proximally, becoming hard, necrotic and excoriated distally ([Images 673-675](#)). Histopathology showed multiple areas of chondrocyte proliferation with minimal differentiation towards cartilage formation, and a diagnosis of chondrosarcoma was made.

11.3.15 No diagnosis

No diagnosis was made in 42 pigeons and doves. Most (28) were feral pigeons that had been culled because they were showing CNS signs. Additional tests were not carried out or were inconclusive, but most were probably suffering from either PPMV-1 infection or head trauma. Other feral pigeons and two woodpigeons were in thin or moderate condition and had fluid intestinal contents of unknown cause, some of which may also have been undiagnosed cases of PPMV-1 infection.

11.3.16 Incidental findings

Histopathological examination of the liver of a woodpigeon submitted in February with confirmed avian pox revealed large numbers of gametocytes of *Leucocytozoon marchouxi* in white blood cells in the liver sinusoids and larger vessels (Images 630-632). Schizonts of *L. marchouxi* and *Haemoproteus columbae* were also detected in hepatocytes. Histopathology was carried out because the liver was enlarged, dark and granular, but the haemoparasites did not appear to be causing any pathology and were considered to be incidental findings. Biting arthropods can be involved in the transmission of avian pox, and in this bird may also have transmitted the haemoparasites.

Tissues from 56 birds were sent to AHVLA Weybridge to be screened for avian influenza viruses, but no positive results were reported. Similarly, tissues from 72 birds were sent to AHVLA to be screened for West Nile virus, but no positive results were received.

11.4 Discussion

11.4.1 General comments

The results of this study showed the importance of infectious diseases in birds of the order Columbiformes, and the potential for the spread of pathogens within wildlife rehabilitation centres. Stocker (2000) proposed that all pigeons and doves should be quarantined for at least 10 days before entering the main wildlife centre, and Chitty (2003) advocated prompt euthanasia of birds with signs suggestive of PPMV-1, severe pox, salmonellosis, chlamydiosis and advanced trichomonosis. Trichomonosis, spironucleosis, PPMV-1 infection and avian tuberculosis are discussed in greater detail below (11.4.2 – 11.4.4).

Pox infection is usually readily recognised by those working with pigeon casualties, and the lesions observed in this study were typical of those described elsewhere. It is interesting to note that cases were confined to woodpigeons, with none observed in feral pigeons or collared doves, suggesting that woodpigeons are especially susceptible to this disease. Infections with other viruses such as adenovirus and circovirus may be less obvious to wildlife rehabilitators but equally, if not more, significant. IBH caused by an adenovirus is not uncommon in racing pigeons in the UK (Pennycott 2008) and can result in necrotising hepatitis, diarrhoea and death, with secondary *E. coli* involvement in some birds. Pigeon circovirus infection is also common in racing pigeons, damaging the bursa of Fabricius and resulting in immunosuppression and secondary infections (Pennycott 2008). The confirmation of these conditions in feral pigeons is therefore not surprising, and almost certainly such viral infections in feral pigeons are greatly under-diagnosed. There is clearly a risk that an infected bird could spread these viruses to other birds in a rehabilitation centre if such a bird was to be admitted. However, routinely screening feral pigeons submitted to rehabilitation centres for the presence of these pathogens would be very difficult, underlining the importance of quarantine measures for pigeon casualties and the value of regular postmortem examinations on birds that have died or been culled.

Hairworms, coccidia and to a lesser extent tapeworms were often found in feral pigeons and woodpigeons in this study, but only infrequently in collared doves. Although in most cases they were considered to be incidental findings, parasite burdens could eventually become significant in birds admitted to wildlife rehabilitation centres, and the findings support the advice by Stocker (2000) and Chitty (2003) that pigeons and doves in wildlife centres should routinely be wormed. Alternatively, regular faecal screening for parasites should be carried out. More unusual was the demonstration of *Ornithostrongylus quadriradiatus* in the small intestine of a woodpigeon with haemorrhagic enteritis, and as an incidental finding in a stock dove. *O. quadriradiatus* can cause diarrhoea and mortality in racing pigeons (Rose and Keymer, 1958), and Harper (1996) considered it to be the most pathogenic of the nematodes found in racing pigeons. *Ornithostrongylus* is not a common finding in racing pigeons in the UK (T. Pennycott, unpublished data), and does not appear to be widespread in feral pigeons, collared doves or woodpigeons.

In addition to spreading pathogens to other birds, pigeons and doves can also be a source of zoonotic pathogens. Chlamydiosis was diagnosed in ten birds; five feral pigeons, four collared doves and a woodpigeon. During the current study only small numbers of birds were screened for chlamydial antigen by PCR, and it is likely that larger numbers were clinically affected or asymptomatic carriers. If such birds were admitted to wildlife rehabilitation centres, they would pose a threat to other birds and also to humans working at the centre. Wildlife rehabilitators must be made aware of these risks and appropriate precautions taken, including the euthanasia of birds suspected to be affected by chlamydiosis. Relatively small numbers of birds had salmonellosis, another potentially zoonotic pathogen, and overall the organism was detected in only 4% of the columbiforms screened. Nevertheless, for the reasons discussed for chlamydiosis, birds with suspected salmonellosis should be euthanased. Although not detected in the current study, *Yersinia pseudotuberculosis* has frequently been isolated from columbiforms such as woodpigeons (Mair 1973) and stock doves (Clapham 1953), and this bacterium can

cause enteric disease in humans. Strains of Shiga toxin-producing *E. coli* O157:H7, capable of causing disease in humans, have been recovered from feral pigeons in Italy (Santaniello et al., 2007). Pigeon faeces provide a good growth medium for the yeast *Cryptococcus neoformans*, an organism found throughout the world (including the UK) and which can cause meningitis in humans, especially those who are immunocompromised (Cogliati 2013; Sloan and Parris, 2014). Immunocompromised humans are also susceptible to infection with microsporidia such as *Encephalitozoon* spp., resulting in a wide range of clinical signs; a study in Brazil found microsporidia in the faeces of 31.3% of feral pigeons tested (Lallo et al., 2012). The potential zoonotic risks presented by pigeons and doves, especially in wildlife rehabilitation centres, should not be underestimated, and should be borne in mind when considering whether or not to admit columbiform birds to rehabilitation centres.

The commonest non-infectious causes of death or culling were trauma (all age groups) and metabolic bone disease in immature birds. Cousquer et al. (2007) took the view that metabolic bone disease was a naturally-occurring problem and that attempts to treat affected birds could interfere with natural selection processes. If treatment was to be attempted, these authors suggested the administration of vitamin D, possibly with additional calcium and exposure to UV light.

11.4.2 Trichomonosis and spironucleosis

Trichomonosis (confirmed or suspected) was the commonest infectious disease of pigeons and doves diagnosed during this study, mostly in collared doves and woodpigeons and especially in the fourth quarter of the year. In view of these findings, there may be merit in routinely administering an antiprotozoal agent to all pigeons and doves admitted to wildlife rehabilitation centres, as suggested by Stocker (2000) and Chitty (2003). Such routine prophylactic medication, alongside strict hygiene measures, would also help to reduce the spread of this pathogen to other birds in the rehabilitation centre.

Motile protozoa with the morphology of *Spironucleus* (*Hexamita*) sp. were demonstrated in the small intestine of four feral pigeons and a woodpigeon. All the birds were thin and had fluid intestinal contents. These organisms are difficult to detect in the carcasses of birds that have been dead for several hours, because the protozoa stop moving when the carcase cools down after death. They may, therefore, have been present but undetected in other carcasses. *Spironucleus* is known to be a cause of diarrhoea and death in racing pigeons and psittacine birds (Harper 1991), turkeys (Wilson and Slavin, 1955) and gamebirds (Swarbrick 1990), but their presence in wild or feral birds in the UK has not previously been reported. It is unknown whether different species of *Spironucleus* are found in different groups of birds (Lloyd et al., 2005), and the role of feral pigeons and woodpigeons as a source of infection for poultry and game birds remains uncertain. Spironucleosis should be included in the differential diagnosis of causes of diarrhoea in casualty pigeons, and microscopy of freshly voided faeces may demonstrate the actively motile protozoa, confirming the diagnosis. The routine administration of antiprotozoal agents to control trichomonosis may also help to reduce the risk of spironucleosis.

11.4.3 PPMV-1 infection and other causes of CNS signs

Many of the pigeons submitted for postmortem examination had shown CNS signs prior to death or euthanasia. Numerous infectious and non-infectious conditions can damage the CNS of columbiform birds, including infections with *S. Typhimurium* and PPMV-1; trauma; thiamine deficiency; secondary hypoglycaemia; and poisoning by chemicals such as organophosphorus and organochlorine compounds, metaldehyde, alphachloralose and monochloroacetate (Pennycott 1996b). Neurological signs associated with *Sarcocystis* sp. have been described in racing pigeons in Germany (Olias et al., 2009) and the USA (Wunschmann et al., 2011). In the current study it was not possible to test samples from every bird for all of the above conditions, but some observations can still be made.

Traumatic lesions to the head were found in some birds and suspected in others, and salmonellosis was diagnosed as the cause of CNS signs in one feral pigeon. *Pasteurella*

multocida can cause CNS signs in turkeys and pheasants (Higgins and Randall 1981), and in the present study this organism was recovered from the brain of three birds showing CNS abnormalities. However no brain histopathology was carried out in these birds and the significance of the isolation of this organism was unclear.

In the absence of obvious traumatic lesions or evidence of salmonellosis, the most likely cause of the CNS signs described in the majority of the remaining feral pigeons was PPMV-1 infection. This disease was subsequently confirmed by virus isolation or serology in 18 feral pigeons, but samples from other birds were not tested or gave inconclusive results, and it is likely that many of the other feral pigeons exhibiting abnormalities of the CNS were also affected by this virus. PPMV-1 has caused a panzootic in racing pigeons and feral pigeons in many countries including the UK (Lister et al., 1986) and has also been recovered from collared doves and woodpigeons in the UK (Anon 2011b; Anon 2011d). The possible involvement of PPMV-1 should therefore be considered in all feral pigeons, collared doves and woodpigeons that have died or been euthanased after showing diarrhoea, weight loss or CNS signs. There is a significant risk that columbiform birds admitted to a wildlife rehabilitation centre might be excreting PPMV-1 that could then spread to other birds in the centre, including birds due to be released, with the potential for further spread of this virus. It is, therefore, essential that hygiene and quarantine measures are routinely enforced to reduce the likelihood that infected birds are admitted to the main part of wildlife rehabilitation centres. This should include the prompt culling of birds considered to be suffering from this disease, and rigorous hygiene and management measures to limit the potential spread of the virus within the centre.

The presence of PPMV-1 in wild birds of the order Columbiformes is also of concern because these birds can and have spread the virus to poultry flocks (Alexander et al., 1985). One consequence of the notifiable disease legislation in Great Britain is the requirement to notify the Animal and Plant Health Agency if Newcastle disease or PPMV-1 is suspected to be present in any captive bird or carcase (Anon 2012c). This

could include wild birds while they are being held captive in a rehabilitation centre, and statutory disease control measures including movement controls might then be placed on the centre. Such measures could significantly interfere with the running of the centre, underlining the need to observe strict quarantine and hygiene measures when rehabilitating birds of the order Columbiformes.

11.4.4 Avian tuberculosis

A wide range of pathological lesions was encountered in birds with avian tuberculosis, presumed to be caused by *Mycobacterium avium*. The lesions observed in the liver, spleen and intestine of woodpigeons in the present study were similar to those described by McDiarmid (1948), and the cutaneous form observed in a woodpigeon and a collared dove resembled that described by Blackmore and Keymer (1969) in a kestrel (*Falco tinnunculus*) and a herring gull. Lung lesions were not described by these authors, but in three woodpigeons in the current study, one lung was almost completely replaced by a large well-circumscribed mass. The pigmented fissured lesions affecting the duodenum of four woodpigeons were reminiscent of the pigmented form of Johne's disease in sheep, caused by *M. a. paratuberculosis* (Clarke and Little, 1996); there may be merit in further exploring possible links between Johne's disease in ruminants and avian tuberculosis in woodpigeons, following up the experimental findings reported by Matthews and McDiarmid (1979) and Collins et al. (1985). One woodpigeon with avian tuberculosis had severe visceral and articular gout, and similar changes in two woodpigeons, one of which had avian tuberculosis, were described by Blackmore and Keymer (1969), who commented that such findings appeared to be rare in wild birds. In the only affected feral pigeon, the mycobacterial mass was in the subcutaneous tissues in the region of the crop, with a fistula tracking from the crop to the mass, suggesting that the pathogenesis differed from that seen in woodpigeons.

No attempt was made in the current study to culture or further identify the mycobacteria associated with the lesions of avian tuberculosis, but the use of molecular typing techniques should be considered in future studies to further our understanding of this

disease in woodpigeons and other birds and animals. These findings serve as a reminder to wildlife rehabilitators and their veterinary advisers of the need to consider the possibility of avian tuberculosis in woodpigeons that are presented in very poor body condition, and in columbiforms with firm nodular swellings on their wings. Not only is the prognosis in such cases very poor, but infected birds may also contaminate the wildlife hospital premises, with the risk of spread of disease to other birds on-site, including those due to be released back to the wild.

Chapter 12

Conclusions

12.1 Introduction

The UK general public takes considerable interest in the well-being of birds and wild animals. This is reflected in the many households that provide supplementary feeding to garden birds and the large number of casualties that are submitted to wildlife rehabilitation centres. In addition, members of the public frequently wish to find out the cause of death of wild birds found dead in their gardens and elsewhere, and wild bird disease surveillance is also of interest to a wide range of governmental and non-governmental organisations. However, as discussed in Chapters 1 and 7, the provision of supplementary feeding to garden birds, and the rescue and rehabilitation of sick or injured wildlife, may not always be in their best interests. Are we “killing with kindness”? In addition, although much wild bird disease surveillance has been carried out in the past eighty years, the results are often not readily available and have not been adequately collated, analysed and interpreted. The purpose of this thesis was to make maximum use and dissemination of surveillance data already gathered, and to explore the implications of the findings for garden bird feeding and wildlife rehabilitation. The research was of an exploratory and descriptive nature, based on in-depth reviews of the available literature and of the findings from wild bird disease surveillance carried out at Ayr Disease Surveillance Centre (DSC) between 1994 and 2013.

The objectives of wild bird disease surveillance at Ayr DSC and of this thesis were listed in 1.4.4 and are repeated here. The population of interest for the purposes of the thesis was defined as *wild birds of the orders Passeriformes and Columbiformes found dead or sick in Scotland between 1994 and 2013*.

- **Objective 1.** Provision of hazard-specific (targeted) surveillance for specified pathogens (*Salmonella* spp., *Escherichia albertii*, *Mycoplasma* spp., avian influenza viruses, West Nile virus) in the population of interest.

- **Objective 2.** Provision of enhanced passive surveillance to increase awareness of, or add to our understanding of, diseases or pathogens known to be endemic in the population of interest.
- **Objective 3.** Provision of early-warning surveillance to detect exotic, re-emerging or new (emerging) diseases or pathogens in the population of interest.
- **Objective 4.** Provision of enhanced passive surveillance to detect accidental or deliberate harm to wild birds in the population of interest, including poisoning, deaths in birds at garden feeding stations, and losses in rehabilitation centres.
- **Objective 5.** Timely reporting of results and trends to stakeholders, including those funding the surveillance and those submitting the carcasses.
- **Objective 6.** Retrospective review of postmortem findings from wild birds from the population of interest, and assignment of up to three diagnoses based on previously-defined diagnostic criteria.
- **Objective 7.** Preparation of a review of diseases reported from wild birds of the orders Passeriformes and Columbiformes in the UK, collation of results from wild bird surveillance at Ayr DSC between 1994 and 2013, and discussion of the significance and implications of the findings.
- **Objective 8.** Provision of data on the antimicrobial susceptibility or resistance of bacterial isolates from the carcasses of finches and sparrows, and from wild bird faeces from garden feeding stations.
- **Objective 9.** Preparation of a collection of images of pathological lesions, parasites and parasite oocysts or eggs from the population of interest, to be made available to those involved with wildlife disease surveillance, diagnosis or treatment.
- **Objective 10.** Preparation of a summary of descriptions of internal parasites and their oocysts or eggs, to aid in the presumptive identification of internal parasites from the population of interest and to be made available to those involved with wildlife disease surveillance, diagnosis or treatment.

This concluding chapter assesses whether these objectives were met, by evaluating wild bird disease surveillance at Ayr DSC in terms of: coverage, representativeness and bias;

outcomes; communication of results; data collection, completeness, correctness and management; and the retrospective collation, analysis and discussion of the results. Comments are made about the antimicrobial susceptibility testing data (Appendix IV), the image collection (Appendix X), and the guide to the presumptive identification of parasites (Appendix III). Final suggestions are presented on the advisability of providing supplementary feeding for garden birds, and on the potential impact of infectious diseases on wild bird rescue, rehabilitation and release. For some pathogens and diseases, the results presented in this thesis represent a starting point only, with much further work required, and the chapter therefore concludes with recommendations about future surveillance and follow-up investigations.

12.2 Evaluation of wild bird disease surveillance (Objectives 1-7)

12.2.1 Coverage, representativeness and bias

The proportion of the population of interest that was sampled (coverage) was necessarily very small, and the non-random selection of carcasses for submission by members of the general public and rehabilitators meant that those carcasses examined would not have been fully representative of the population of interest. Similarly, for birds other than finches, 88% of carcasses originated from the south of Scotland and only 12% from the north, indicating poor geographic coverage and representativeness. As a result, attempts to estimate the prevalence of individual pathogens or conditions in non-finches in different parts of Scotland would be prone to selection bias. The non-random selection of tests conducted on individual carcasses, and the low sensitivity of some of the screening tests carried out, would also have resulted in bias.

However, the main purpose of the wild bird disease surveillance was not to determine the prevalence of diseases or pathogens in different types of wild birds. Instead, the aim was to highlight trends in disease occurrence, to increase awareness of the different diseases likely to be encountered by those involved with wildlife rehabilitation and surveillance, and this aim was achieved. Better coverage and representativeness was

achieved for finches due to the ease of posting small carcasses, with 40% of carcasses originating from the north of Scotland and 60% from the south. The extended period of surveillance (twenty years) also added to the value of the data. Accordingly, in the case of salmonellosis, *Escherichia albertii* bacteraemia and trichomonosis in finches, the large number of carcasses examined and improved coverage and representativeness meant that bias was reduced, allowing more in-depth analysis of the results (Chapters 4-7).

12.2.2 Outcomes

A summary of the outcomes of disease surveillance in 2048 wild birds of the orders Passeriformes and Columbiformes which were examined at Ayr DSC between 1994 and 2013 is included at Appendix VIII. Despite the constraints discussed in 12.2.1, 42 endemic conditions were diagnosed, raising awareness of these diseases and consolidating the literature already available on these topics. Increased information was obtained about 14 of these endemic conditions or pathogens, extending the currently available literature. Examples include the importance of helminthosis in different species of corvids; deaths from coccidiosis, helminthosis and aspergillosis in blackbirds; a respiratory syndrome in corvids associated with mixed pathogens, including *Mycoplasma gallisepticum*-like organisms, *Pasteurella multocida* and *Chlamydia psittaci*; the possible role of coal tits as carriers of *S. ornithocola*; and the identification in feral pigeons of conditions previously well-recognised in racing pigeons, such as inclusion body hepatitis, circovirus infection and spironucleosis.

The outcomes also highlighted variation in disease trends between different groups of wild bird. For example, salmonellosis was not a problem in blackbirds, thrushes, robins or corvids, but was seen occasionally in pigeons and frequently in finches and sparrows; helminths were commonly found in blackbirds, corvids and pigeons, but rarely in finches or sparrows; and *E. albertii* bacteraemia was confined to finches, especially siskins and greenfinches. Information about endemic diseases will help those working at wildlife rehabilitation centres, by improving diagnosis, triage, treatment, biosecurity and

other working practices, and will provide baseline data for those carrying out routine wild bird disease surveillance.

One re-emerging disease, namely salmonellosis in garden birds, came to prominence and then declined during the surveillance period. Salmonellosis was diagnosed in approximately 350 garden birds, and this large number of confirmed cases over a prolonged period of time permitted analysis of regional variation in phage types, seasonal patterns, nature of the lesions, and distribution by age and sex (Chapter 4). This has increased our understanding of the epidemiology of this disease and helped formulate advice to reduce the impact of salmonellosis in garden birds, benefiting garden birds and members of the public providing supplementary feeding.

In 2005, wild bird disease surveillance at Ayr DSC recorded the first confirmed case of *Trichomonas gallinae* infection of finches in the UK, a condition first seen in 2000 in the USA. This disease, exotic to the UK, was subsequently suspected or confirmed in approximately 370 finches from Scotland during the study period. The established network of Scottish sites submitting finch carcasses for necropsy allowed conclusions to be drawn regarding its origin and spread within Scotland. In addition, the failure to detect trichomonosis in the large number of finch carcasses examined prior to 2005 supported the view that this was a novel condition in finches in Scotland. *Escherichia albertii* bacteraemia in finches was another new disease identified during the surveillance period, and was diagnosed in approximately 150 finches in Scotland. As noted for salmonellosis, such large numbers of birds with trichomonosis and *E. albertii* bacteraemia permitted further analysis by species of bird, regional variation, and distribution by age and sex (Chapters 5 and 6). Comparisons between these three infectious diseases in different species of bird were also possible, increasing our understanding of their epidemiology and enabling the provision of advice to reduce their impact in garden birds (Chapter 7). Awareness of these three conditions will benefit wildlife rehabilitators and their veterinary advisers at the initial examination stage of

casualties, and highlights the importance of measures to limit the spread of these diseases within rehabilitation centres.

Three other organisms identified in wild birds elsewhere in the world, but reported for the first time in the UK as part of disease surveillance at Ayr DSC, were the avian gastric yeast *Macrorhabdus ornithogaster* (“megabacteria”) in greenfinches and a waxwing, *Mycoplasma sturni* in blackbirds, starlings and corvids, and *Ornithonyssus* sp. mites in corvids. Although the significance of *M. ornithogaster* and *M. sturni* remains unclear, knowledge that they occur in certain species of wild birds is of benefit to those involved with wild bird surveillance. If the *Ornithonyssus* sp. mite found in corvids, sometimes in very large numbers, is *O. sylviarum* (the northern fowl mite), infested corvids would pose a threat to poultry. There is also a risk that this blood-sucking mite could spread to other species of birds in rehabilitation centres, with a detrimental effect on their health. Wild bird rehabilitators and their veterinary advisers must, therefore, be made aware of this risk and take appropriate action.

Two new diseases were identified in choughs as part of this study, namely developmental abnormalities of the eye and sometimes brain of nestlings and fledglings, and significant helminthosis caused by spirurid gizzard worms and intestinal thorny-headed worms. The numbers of breeding choughs in Scotland have declined in recent years, prompting investigations to determine the causes and develop proposals to reverse the decline. The identification of developmental abnormalities in young choughs, most likely inherited through an autosomal recessive allele and made more significant by inbreeding, will help to formulate conservation plans for the future. Awareness of novel forms of helminthosis, not previously described in choughs and possibly reflecting changes in the natural diet of the birds, will be of benefit to those monitoring the effects of mitigation strategies such as the supplementary feeding of choughs.

Three conditions were encountered for which no satisfactory aetiological agent could be identified: a non-suppurative encephalitis in fledgling starlings and house sparrows;

enteritis and hepatitis in two blackbirds associated with schistosome-like eggs; and a necrotic oesophagitis of unknown cause in five chough nestlings. Material from these cases has been retained and it is hoped that improved diagnostic techniques in the future will establish the causes. If the organisms detected in blackbirds are eggs of trematodes of the family Schistosomatidae, they may play a role in cercarial dermatitis of humans, and further identification of these schistosome-like eggs is required.

Samples from over 600 birds were collected and submitted to AHVLA/APHA for screening for highly pathogenic avian influenza virus (HPAIV) and from over 500 birds for West Nile virus (WNV). These two zoonotic pathogens are exotic to UK wildlife, and no positive results were obtained. The results contributed to national targeted surveillance and helped policy-makers assess the level of risk posed by these viruses. Nevertheless, the outcomes of wild bird disease surveillance reinforced awareness that wild birds may be sources of other zoonotic pathogens, including *S. Typhimurium*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Chlamydia psittaci*, and possibly *E. albertii*. Personal hygiene by those handling live wild birds, carcasses, feeders or cages contaminated by wild bird faeces etc. is therefore essential.

12.2.3 Communication of results

Members of the general public or rehabilitation centres that submitted carcasses were sent the results of the postmortem examination in a timely fashion, with updates as required. Significant findings were summarised in the monthly reports of SACCVS in the Veterinary Record, in quarterly reports to the GB Wildlife Disease Surveillance Partnership and in annual reports to the OIE (when such options were available). Quarterly summaries were sent to major funders such as the Dulverton Trust, the Garden Bird Health initiative (GBHi) and Scottish Government. Numerous presentations on wild bird diseases were given to the general public and scientific community. Newsletters, fact sheets and web pages were produced, and the author of this thesis was a sole or joint author of 23 letters, short communications and peer-reviewed papers about diseases in wild birds. Therefore, communication to stakeholders, the general

public in Scotland and the scientific community was considered to be good. Nevertheless, publication of this thesis on-line would substantially increase the number of people having access to the information.

12.2.4 Data collection, completeness, correctness and management

During the course of the surveillance period, several different necropsy protocols were in place, depending on the requirements of the funders and the resources available (Appendix IX). In some cases protocols for sample collection were followed, for example when carrying out targeted surveillance for HPAIV and WNV, or when carrying out postmortem examinations of garden birds under the GBHi (Appendix IX). In an ideal world, one protocol would have been used throughout the entire surveillance period, but the evolving nature of the surveillance and funding meant that such an approach was not possible. The degree of autolysis of some carcasses also influenced the range of diagnostic tests performed. As a result, data collection was not consistent throughout the surveillance period, and some examinations that would have been useful (e.g. wet preparations from the crop and intestine for evidence of parasites) were not conducted in all birds.

Another area in which data collection was incomplete was examination for haematozoa. Microscopy of stained blood smears for the presence of haematozoa was not carried out due to insufficient time resources and expertise. Some of the limitations in interpreting avian blood smears were summarised by Cooper and Anwar (2001). These included the need for specific training in making and interpreting blood smears; the importance of examining several different parts of the blood smear and for adequate lengths of time; and variability in the number of detectable parasites depending on the level or periodicity of infection. In addition, it has been suggested that there is little value in collecting samples from carcasses unless they are reasonably fresh (Peirce and Bevan, 1977). The decision was therefore taken at the outset of the study that examination for blood parasites would not be carried out. However, recent years have seen the development of nested PCRs and real-time PCRs for the detection of avian

haemoparasites. These techniques are believed to be more sensitive and faster than traditional microscopy, permitting the screening of large numbers of samples without the need for specialised training in making and interpreting blood smears (Bell et al., 2015), and their use should be considered in future studies.

Despite the above limitations, the data that was collected was of high quality. Investigations at Ayr DSC and other laboratories within SACCVS were carried out in accordance with Standard Operating Procedures, and during the study period these laboratories achieved ISO 17025 accreditation for some procedures. The majority of the testing carried out by outside organisations was similarly covered. In addition, a comprehensive set of diagnostic criteria (Appendix II) was used to establish up to three diagnoses in each submission, and guidelines were followed when identifying internal parasites (Appendix III), thus ensuring a consistent approach to diagnosis. When measuring coccidial oocysts or helminth eggs (Chapter 2 and Appendix III), the accuracy of the measurements was checked using a calibrated micrometer slide on every occasion.

Each carcase was given a unique submission reference number and details entered on a computer-based Laboratory Information Management System (LIMS). The results of laboratory testing were also entered in LIMS, as was the written postmortem report. This LIMS system was designed for recording data from farm livestock and pets, and complied with Good Laboratory Practice standards. Although insufficient fields were available to capture all required data, this was partially addressed by recording additional details as free text in the final report section, permitting retrospective interrogation of data. This was complemented by the retention of the original paperwork for a minimum of seven years.

In summary, the data that were collected and recorded were of high quality, but data from some carcasses were incomplete. This inconsistency could be addressed in the future if greater time and other resources were made available for wild bird disease

surveillance. If large-scale wild bird surveillance is to continue in the future, there would be merit in devising and using a LIMS system specifically tailored for that purpose. Nevertheless, the system used during the study period ensured that the data recorded were assigned to the correct carcass and were backed-up and stored for future analysis.

12.2.5 Retrospective collation, analysis and discussion of results

Key aspects of disease surveillance are the collation, analysis and interpretation of the data collected (see Table 1.2). Although parts of these elements were addressed in various communications to stakeholders, overall analysis and interpretation on a larger scale was previously limited. A major intention of this thesis was to make better use of data already gathered, and the results and discussion sections in Chapters 3-11 indicate that this has been achieved. In-depth reviews of disease conditions previously found in UK wild birds were also conducted as part of this thesis, helping to put the findings into context. Publication of the thesis on-line will further disseminate the results and discussion, and provide those working in the fields of wildlife rehabilitation and wildlife disease surveillance with comprehensive summaries of diseases found in UK birds of the orders Passeriformes and Columbiformes.

12.3 Data on antimicrobial susceptibility or resistance (Objective 8)

Antimicrobial resistance (AMR) is a major problem in humans and livestock, and wild birds can act as sentinels for the emergence of new AMR organisms (see Chapter 1). The results from the current study, presented in Appendix IV and summarised in Chapters 3-5, showed that for non-lactose fermenting (NLF) bacteria of the Enterobacteriaceae family, wild finches and sparrows were unlikely to be significantly involved in the transfer of AMR bacteria or AMR genes to humans or livestock. Over 500 isolates of *Salmonella* spp., *Escherichia* spp. and *Yersinia* spp. from finch or sparrow carcasses were tested using the disc diffusion method, and only three isolates showed resistance to more than one different antimicrobial group – β lactam and fluoroquinolone resistance in *E. albertii* from a siskin, and β lactam and tetracycline resistance in *Escherichia* spp. from a house sparrow and a chaffinch. However, as

outlined in Appendix IV, each isolate was not tested against every antimicrobial and differences in interpretative criteria occurred in different time periods. Repeat examination of stored isolates, using molecular tests that detect a wide range of the genes that confer AMR, is therefore recommended to give greater coverage of different types of AMR and their genetic basis. When molecular testing was carried out on isolates of *Staphylococcus aureus* from wild birds, an isolate from a chaffinch was found to have a novel form of the *mecA* gene that confers resistance to meticillin, the first time that MRSA had been found in a wild bird (see Chapter 3). Similar targeted testing may reveal other important or novel forms of AMR in wild birds.

The methodology used to recover bacteria from finches and sparrows (Chapter 2) did not include the use of media that selected for bacteria exhibiting AMR, for example media to detect extended-spectrum β lactamase-producing (ESBL) bacteria. When this approach was employed in gulls in Scotland in 2012, ESBL-producing *E. coli* was detected in 13 out of 30 (43.3%) gulls tested, and four of the 13 ESBL-producers from gulls were resistant to four or more unrelated antimicrobials (SRUC 2015). Incorporating selective AMR media into future testing protocols is recommended, to improve screening for important forms of AMR such as ESBL and carbapenemase production.

12.4 Images of pathological lesions and parasites, and presumptive identification of internal parasites (Objectives 9 and 10)

A collection of over 700 images of pathological lesions, parasites and their eggs or oocysts, taken from wild birds of the orders Passeriformes and Columbiformes during the twenty years of disease surveillance, is included as Appendix X to this thesis. Although it was not possible to collect images of every condition or parasite, the collection is nevertheless extensive and will be of major assistance and interest to those training or working in wild bird disease surveillance, diagnosis and treatment. It is the intention to provide as wide access to the images as possible, and in the future it would

be desirable to combine this collection with images assembled by other wildlife pathologists and parasitologists.

For similar purposes, a guide to the presumptive identification of some of the internal parasites encountered is included as Appendix III, with summaries of the appearance and dimensions of the parasites and/or their eggs or oocysts. It is recommended that this database be expanded in the future, to increase the number of measurements collected and the range of parasites covered. However, even in its present form, and in combination with the images in Appendix X, this appendix will be of value to those working with parasitic infections of wild birds, including those working in rehabilitation centres who regularly screen faecal samples for evidence of coccidiosis and helminthosis.

12.5 Should we provide supplementary feeding for garden birds (Objective 7)?

Some of the arguments for and against feeding garden birds were discussed in Chapter 1, and the significant role played by supplementary feeding in the transmission of infectious diseases such as salmonellosis, *E. albertii* bacteraemia and trichomonosis was examined in detail in Chapter 7. Should we feed garden birds? Having considered the evidence presented, it is the view of the author of this thesis that the advantages of feeding garden birds outweigh the disadvantages, provided that the feeding is carried out in a responsible and sustainable manner.

In my opinion, feeding garden birds should be regarded and promoted as a form of *environmental stewardship*, defined by Wikipedia as *the responsible use and protection of the natural environment through conservation and sustainable practices* (https://en.wikipedia.org/wiki/Environmental_stewardship Accessed 05/01/16). As has been seen with the emergence and spread of finch trichomonosis, the provision of supplementary feeding to garden birds can have significant detrimental effects. It would be *irresponsible and poor use of the environment* to feed garden birds if this resulted in

outbreaks of infectious disease. Householders who encourage garden birds to visit an artificially-created and potentially hazardous environment by providing supplementary feeding must be prepared to take some *responsibility* for the health and welfare of the birds they are attracting. *Protection of the natural environment*, by taking measures to reduce a build-up of pathogens in and around bird feeders, will be crucial if infectious disease is to be limited. The other key elements of environmental stewardship are *conservation* and *sustainability* and, as seen with trichomonosis in greenfinches, infectious diseases spread at garden bird feeders can have a major impact on the *conservation* and *sustainability* of some species at a population level. Feeding garden birds should only be undertaken if the householder is prepared to accept the role of *environmental steward* and the responsibilities that go with this role, and this is a message that organisations such as RSPB and BTO, and companies marketing wild bird food, should vigorously promote.

To feed garden birds in a responsible and sustainable fashion, adopting the principles of environmental stewardship, the following “top-ten” actions are recommended by the author of this thesis:

1. The hygiene measures at feeding stations as described in 7.8.1 are applied. The frequency of cleaning and disinfection will depend on the scale of feeding.
2. Food should not be provided every day, but instead there should be periodic breaks in provisioning.
3. Feeding should be stopped for at least three weeks if several sick or dead birds are observed at or near the feeding station within a short period of time, and attempts made to establish the cause of the mortality.
4. The quantity of food provided should be consumed within 48 hours and should not encourage large numbers of birds to feed within a small area. If necessary, the food should be divided among multiple feeding stations.
5. The type and quality of the food should be as advised by organisations such as RSPB, BTO and GWH.
6. Feeding during the summer months should be restricted or stopped.

7. Be aware of the risks involved in attracting less-common species of birds to feeding stations, and change the feeding programme if necessary to protect such vulnerable birds.
8. If possible, provide natural food sources in gardens, in addition to or instead of artificial feeding.
9. Be prepared to discuss feeding strategies, and any problems encountered, with immediate neighbours who may also be feeding the birds.
10. Be aware that some diseases of garden birds could spread to humans (especially children) and pets, and therefore observe appropriate personal hygiene measures.

12.6 Disease implications for wildlife rehabilitation and release (Objective 7)

The potential hazards posed by infectious diseases in wildlife rehabilitation centres were discussed in Chapter 1. During the current study, numerous infectious conditions were diagnosed in wild birds, sometimes within rehabilitation centres, reinforcing the need for wildlife rehabilitators and their veterinary advisers to be aware of such diseases. Among the pathogens or conditions identified were: salmonellosis, trichomonosis and *E. albertii* bacteraemia in small garden birds (Chapters 4-7); coccidiosis, helminthosis and mycotic pneumonia in blackbirds (Chapter 8); fledgling CNS disorder of unknown cause in house sparrows and starlings (Chapters 3 and 8); avian pox in house sparrows, starlings and corvids (Chapters 3, 8 and 9); a multifactorial respiratory disease, helminthosis, *Ornithonyssus* sp. mites and *Cnemidocoptes* sp. mites in corvids (Chapter 8); and avian pox, avian tuberculosis, PPMV-1, IBH, chlamydiosis, salmonellosis, circovirus infection, trichomonosis, spironucleosis and helminthosis in pigeons and doves (Chapter 11).

Given the ease with which some pathogens could be transferred among birds in rehabilitation centres and after release, and in some cases to humans or livestock, the advantages and disadvantages of attempting wildlife rehabilitation and release may need to be re-evaluated. Just as responsible and sustainable feeding of garden birds was advocated in 12.5 above, responsible and sustainable working practices must be adhered

to at rehabilitation centres, otherwise infectious diseases could significantly affect the health and welfare of the wild birds. Such measures may include: greater selectivity of species or individuals in which rehabilitation will be attempted; enhanced segregation of different groups of birds to limit the spread of pathogens to unusual hosts; improved hygiene measures; and routine postmortem examinations or other laboratory testing to monitor for infectious diseases. A requirement for rehabilitation centres to be licensed, as outlined in Chapter 1, would go some way to ensuring that these measures are adhered to and that birds in rehabilitation centres receive their legal protection from pain, suffering, injury and disease. When discussing disease transmission at garden bird feeding stations (Chapter 7), key control actions were avoiding a build-up of pathogens in the environment, reducing intra-species contacts, and reducing inter-species contacts; the same three factors also apply to disease control in rehabilitation centres.

At the time of writing this concluding chapter (January 2016), an outbreak of avian influenza caused by low pathogenic subtype H5N1 virus occurred on a poultry farm in the east of Scotland, requiring the culling of 40,000 breeding chickens (<http://news.scotland.gov.uk/News/Avian-influenza-case-2128.aspx>, accessed 12/01/16). This outbreak occurred approximately 15 miles from a large wildlife rehabilitation centre, and although there was no reason to suspect that the centre played any role in the occurrence of avian influenza in poultry, the incident reinforces some of the concerns expressed in this and other chapters.

12.7 Pathogens and diseases of birds of the orders Passeriformes and Columbiformes - further work required

During the study period, several pathogens or conditions were encountered that require continued surveillance or follow-up investigations to determine their aetiology or significance. Among the most important areas of further work are:

- Continued surveillance for *Salmonella* spp., *E. albertii* bacteraemia and trichomonosis, in order to detect changes in presentation of these conditions (Chapters 3-7).

- Field studies to determine the precise role of supplementary feeding in incidents of salmonellosis, *E. albertii* bacteraemia and trichomonosis in finches (Chapters 3-7).
- Continued surveillance in tits for pox infection and *S. ornithocola* infection, to monitor the spread of pox in tits and to determine the possible role of coal tits as carriers of *S. ornithocola* (Chapter 3).
- Further investigation of virulence genes found in *E. albertii* with the API-20E profiles 4144102 and 5144102, and in sorbitol-fermenting strains with the profiles 4144502 and 5144502 (Chapter 5).
- Greater use of PCR for *T. gallinae*, to detect subclinical infections and mixed infections in birds with salmonellosis or *E. albertii* bacteraemia (Chapters 6 and 7).
- Identification of the schistosome-like eggs detected in dunnocks, house sparrows and blackbirds, by more detailed necropsies and molecular techniques (Chapters 3 and 8).
- More detailed investigations into the cause of fledgling CNS disorder in house sparrows and starlings, including the possible role of *Sarcocystis* sp. (Chapters 3 and 8).
- Use of PCR technology to screen birds for haemoparasites (Chapters 3, 8, 9, 10 and 11).
- More detailed investigations into the cause of necrotic oesophagitis in nestling choughs (Chapter 9).
- Further investigations into the causes of corvid respiratory syndrome, including the possible involvement of *Chlamydia suis* (Chapter 9).
- Identification to species level of the spirurid gizzard worms found in choughs, to determine possible intermediate hosts and dietary implications (Chapter 9).
- Identification to species level of the *Ornithonyssus* sp. mites detected in corvids, to assess the potential for spread to free-range poultry (Chapter 9).
- Using culture and molecular techniques, further identification of the mycobacteria causing avian tuberculosis in woodpigeons, to establish if infected birds might play a role in transmitting Johne's disease to ruminants (Chapter 11).

- Greater use of selective media and molecular testing for AMR bacteria (Appendix IV).

12.8 And finally.....

This thesis focused on diseases of wild birds of the orders Passeriformes and Columbiformes, including the necropsy results from 2048 birds. The findings have made a substantial contribution to our knowledge and understanding of diseases in birds of these orders. However, as indicated in Chapter 2, results and images from another 1018 birds of other groups (raptors, seabirds, waterfowl and “others”) were collected during the twenty-year surveillance period. Given time and resources, it would be invaluable to collate and analyse these findings in the same manner as for Passeriformes and Columbiformes, and expand the collection of images and parasite descriptions.

During the twenty years of the study period, a multitude of molecular diagnostic techniques were developed by numerous institutes throughout the world, and no doubt the range of tests will continue to grow. These molecular diagnostic tests have considerably enhanced our understanding of many infectious conditions. Nevertheless, these sophisticated tests complement but do not replace good, old-fashioned, gross pathology and basic diagnostic tests. For wild bird disease surveillance to continue in the future, it will be essential that training and resources for these basic skills are made available. The contents of this thesis and appendices can, hopefully, contribute to this training.